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Mutagenic Activity and Chemical Characterization for the Palo Alto Wastewater Reclamation and Groundwater Injection Facility

Stanford Univ., CA

Prepared for

Health Effects Research Lab.
Cincinnati, OH

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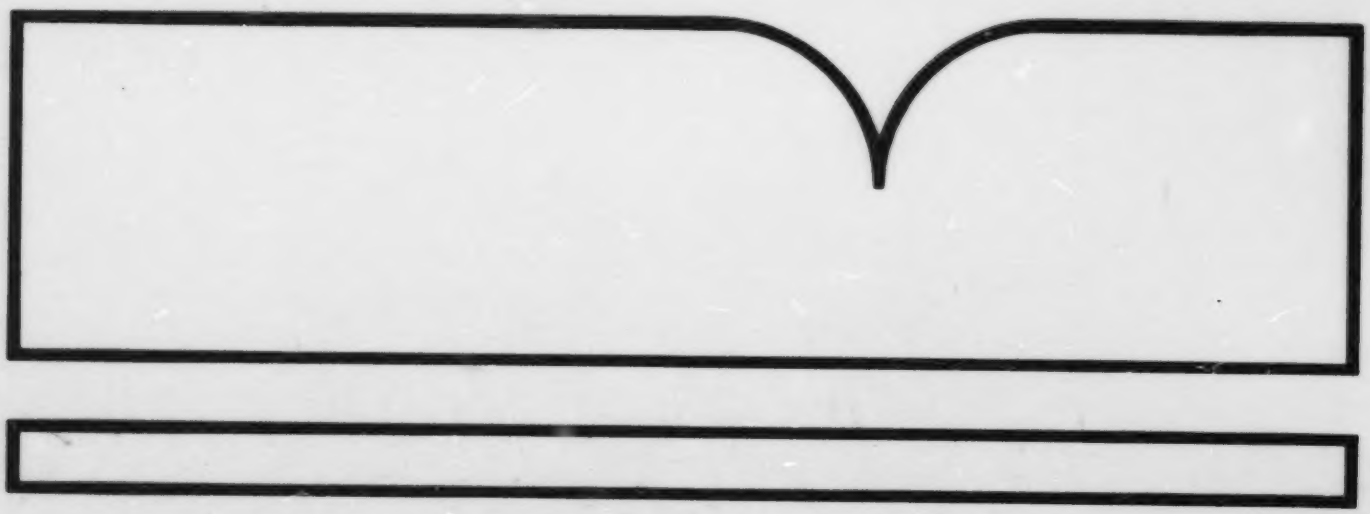

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16. ABSTRACT

At the Palo Alto Reclamation Plant 0.044 m^3/s (1 mgd) of secondary effluent is reclaimed through a series of wastewater treatment processes. Mutagenic activity was consistently found to be present in the secondary treated municipal wastewater influent to the Reclamation Facility. This activity was not reduced significantly by high lime treatment, air stripping, recarbonation, or ozonation, even though these processes did remove a portion of the overall organic content of the wastewaters and many of the volatile organic compounds. Activated-carbon adsorption was effective in removing mutagenic activity to such a degree that mutagenic activity could not be found in water used for injection or that taken from monitoring wells. Clorination resulted in an increase in mutagenic activity. A laboratory study demonstrated that this increase in activity would not result if chlorine dioxide rather than chlorine were used for disinfection. Chlorination resulted in the production of a significant concentration of non-purgeable, but otherwise unidentified, chlorinated organic compounds, as well as trihalomethanes. Formation or removal of such compounds did not seem to correlate with changes in mutagenic activity. Air stripping by the fountain-spray system was most effective in removing volatile organic compounds, while activated carbon was responsible for removals obtained for most other organic materials.

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MUTAGENIC ACTIVITY AND CHEMICAL CHARACTERIZATION FOR THE PALO ALTO WASTEWATER RECLAMATION AND GROUNDWATER INJECTION FACILITY

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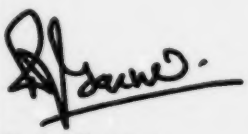
FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare to the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

In this report, the mutagenic activity and chemical constituents at various stages of treatment at the Palo Alto Reclamation Plant are evaluated. Relating adverse biological effects to quality of the wastewater may provide a better understanding of contaminants to be removed and treatment processes required.



R.J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

This study on chemical characterization and the presence of mutagenic activity in municipal wastewater after various stages of advanced wastewater treatment was conducted as part of a larger overall project supported by the Environmental Protection Agency and concerned with wastewater reclamation in Palo Alto, California. The Palo Alto Reclamation Plant is owned by the Santa Clara Valley Water District and is operated for them by the staff of the Palo Alto Water Quality Control Plant. At this facility 0.044 m^3/s (1 mgd) of secondary effluent is reclaimed through a series of processes including high lime treatment, aerator-fountain spraying, single-stage recarbonation, ozonation, mixed-media filtration, activated-carbon adsorption, final chlorination, and storage. A portion of the reclaimed water is injected into a series of wells to serve as a barrier against the intrusion of sea water from San Francisco Bay. The overall study is concerned with advanced wastewater reclamation and the movement and fate of toxic substances after ground-water injection, and this particular study was directed towards monitoring the presence of organic materials which may be of health concern.

Although many specific organic compounds have been identified in reclamation plant effluents, there are many additional compounds that are not readily measured by currently available analytical methods. One method proposed to screen mixtures of unidentified organic compounds to determine possible health significance is the bacterial mutagenicity assay developed by Dr. Bruce Ames, and the potential of this test was explored in this study.

A fraction (averaging about 12%) of the organic material present in water samples after various stages of treatment was removed by passage through a combination of XAD-2 and XAD-7 resins, extracted from the resins with acetone, and after evaporating to dryness and redissolving in DMSO, was subjected to mutagenic assay using bacterial strains TA98, TA100, TA1535, TA1537, and TA1538. In addition, the samples were subjected to several different gas chromatographic and collective parameter analyses to characterize the organic materials present.

Mutagenic activity was consistently found to be present in the secondary treated municipal wastewater influent to the Reclamation Facility, especially to strains TA98 and TA1535 with S9 activation. This activity was not reduced significantly by high lime treatment, air stripping, recarbonation, or ozonation, even though these processes did remove a portion of the overall organic content of the wastewaters and many of the volatile organic compounds. However, activated-carbon adsorption was very effective in removing mutagenic activity, in fact to such a degree that mutagenic activity could not be found in water used for injection or that taken from monitoring wells.

Chlorination of the secondary treated wastewater resulted in an increase in mutagenic activity, especially to strain TA1535. A laboratory study confirmed this field observation and also demonstrated that this increase in activity would not result if chlorine dioxide rather than chlorine were used for disinfection.

The extracts used for mutagenic analyses contained a broad range of fatty acids, phthalates, aromatic compounds, and several unidentified bromine-containing compounds. Because of the complex mixture and the limited effort this study represents, no better resolution of the compounds responsible for the measured mutagenic activity was obtained.

The efficiency of the individual treatment processes for removal (or formation) of a wide range of specific organic compounds and group parameters was measured and are summarized in this report. Chlorination results in the production of a significant concentration of non-purgeable, but otherwise unidentified chlorinated organic compounds, as well as trihalomethanes. Formation or removal of such compounds did not seem to correlate with changes in mutagenic activity. Air stripping by the fountain-spray system was most effective in removing volatile organic compounds, while activated carbon was responsible for removals obtained for most other organic materials.

Future work should be directed towards evaluating further the organic material remaining in reclamation plant effluent, as most of this material remains uncharacterized. In addition, the nature of compounds or mixtures of compounds responsible for mutagenic activity should be determined. The use of the Ames mutagenic analysis for judging the health risk of reclaimed wastewater should be further evaluated.

This report was submitted in partial fulfillment of Research Grant No. R-804431 by Stanford University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period July 30, 1978 through March 31, 1980.

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SECTION 1

INTRODUCTION

Water reclamation and reuse are becoming more important as increasing pollution and water usage reduces the supply of clean water available for beneficial uses. Advanced wastewater treatment processes have been developed to protect receiving waters and to reclaim wastewater for a variety of purposes. Although not currently intended for domestic use, some treated wastewater is also indirectly used at present as a source of drinking water in some communities.

The nature of wastewater is dependent upon the types of industrial and municipal wastes introduced into the sewerage system. Wastewaters may contain a variety of toxic compounds, some of which may not be altered or removed during treatment, and some may be formed during the treatment process itself (1-8).

While today many domestic water supplies contain a small proportion of treated wastewater from upstream discharges, the direct use of reclaimed wastewaters as part of a municipal drinking water supply is not currently permitted. Before this is allowed, the concern over the potentially harmful effects of contaminants remaining in the water must be alleviated. Although research aimed at the identification and quantification of trace materials in water constitutes an active area of environmental chemistry, little is known about the long-term, or chronic, effects of exposing a population to trace levels of these dissolved impurities over a time period of many years. There is a reasonable probability that many of these compounds are carcinogenic, and epidemiological studies do exist which tend to show a degree of correlation between the increased incidence of several forms of cancer and polluted surface waters serving as potable water sources (9-12), as well as to chlorinated drinking water (13,14).

There have been several reports documenting the presence of mutagenic substances in drinking water (15-17) as well as reclaimed wastewater (18). These investigations relied chiefly upon a bacterial mutagenicity assay, the Ames Test (19-21), to detect the presence of mutagens. There is some evidence that many compounds which are bacterial mutagens, as determined by the Ames Test, are also carcinogenic to animals. Thus, this relatively simple test may provide some indication of the presence of potentially harmful substances in water.

This study on the presence of bacterial mutagens in reclaimed wastewater at Palo Alto, California, was instigated as a result of a previous study by

Rappaport et al. (18), who evaluated the presence of such mutagens in samples taken from six different California municipal wastewater treatment facilities. For two of the plants, including the one at Palo Alto, he reported that the mutagenic activity was intensified during the course of the treatment process. In the study reported here, the mutagenic activity at the Palo Alto Reclamation Plant was evaluated in more detail on samples taken after various stages of treatment so that their effect in either producing or reducing mutagenic activity could be better evaluated. In addition, chemical analyses were conducted on collective parameters, such as chemical oxygen demand, total organic carbon, and total organic halogens, as well as on a wide range of specific organic compounds, in order to determine if any correlation with mutagenic activity existed.

Special studies were conducted on the formation of mutagenically active compounds during the chlorination process, and a comparison was made with an alternative disinfectant, chlorine dioxide. In addition, an evaluation was made of the reduction in mutagenic activity during passage of the reclaimed water through an aquifer following groundwater injection.

SECTION 2

CONCLUSIONS

1. Positive mutagenic activity to strains TA98 and TA1535 with S9 activation was consistently measured in the secondary treated wastewater influent to the Palo Alto Reclamation Facility in California.

2. Chlorination of the secondary treated influent resulted in an increase in mutagenic activity, especially to strain TA1535.

3. Laboratory disinfection studies confirmed that mutagenic activity of the secondary treated wastewater increased following chlorination, but would not result had chlorine dioxide disinfection been used.

4. Subsequent processes of high lime treatment, stripping, recarbonation, and ozonation had little effect in reducing mutagenic activity, although in combination these processes were effective in removing many volatile organic constituents of health concern.

5. Activated-carbon adsorption was very effective in reducing mutagenic activity as well as in reducing the organic content of wastewater as measured by the collective parameters of chemical oxygen demand (COD), total organic carbon (TOC), and total organic halogens (TOX).

6. Because of the effectiveness of activated-carbon adsorption in reducing mutagenic activity, few samples of the reclaimed wastewater used for groundwater injection contained positive mutagenic activity.

7. Positive mutagenic activity was not found in any samples taken from the groundwater monitoring wells.

8. An average of only about 12% of the organic material present in the samples analyzed for mutagenic activity was contained in the XAD-acetone extract concentrates; for this reason much of the organic material and all of the volatile organic compounds were not evaluated for mutagenic activity in this study.

9. Fatty acids, phthalates, several aromatic compounds, and several unidentified bromine-containing compounds were present in the XAD-acetone extracts used for mutagenic analysis.

10. The Palo Alto Reclamation Facility is effective in reducing the concentration of a wide range of specific organic compounds and mutagenic activity contained in the secondary treated influent wastewater; however, several materials remain in the reclamation plant effluent at low concentration, and mutagenic activity was detected in some samples.

SECTION 3

RECOMMENDATIONS

1. Procedures should be developed for concentration of organic materials contained in water so that a larger percentage of the materials present can be evaluated for mutagenic activity.

2. Further efforts should be taken to separate and identify the particular compounds responsible for mutagenic activity in wastewater samples.

3. Methods should be developed to reduce the toxic effect of some organic concentrates to strains used for mutagenic testing.

4. Additional efforts are desirable to evaluate the potential of disinfectants other than chlorine which tends to increase mutagenic activity.

5. Animal studies are desirable in order to help evaluate the health significance of positive mutagenic activity in reclaimed wastewaters.

SECTION 4

PALO ALTO RECLAMATION PLANT, INJECTION SYSTEM, AND SAMPLING LOCATIONS

The Palo Alto Reclamation Plant is owned by the Santa Clara Valley Water District (SCVWD) which has the responsibility for the surface and groundwater resources of Santa Clara County, California. Demand has exceeded the supply of local water available and the SCVWD has had to import water from other basins. Since the supply of external water is limited, the District has been evaluating wastewater reclamation to supplement its water supply. The Palo Alto Reclamation Facility was built to help in this evaluation.

The Palo Alto Reclamation Facility is located adjacent to San Francisco Bay on the site of the Palo Alto Regional Water Quality Control Plant. This activated-sludge plant provides secondary treatment to 1.5 m^3/s (35 mgd) of wastewater from the local community, and provides the influent water to the Reclamation Plant. The Reclamation Plant has an overall capacity of 0.088 m^3/s (2 mgd) but generally is operated at a flow rate of 0.044 m^3/s (1 mgd). The plant includes the following processes, as illustrated in Fig. 1: high-lime treatment, an aerator-fountain spray for ammonia removal, single-stage recarbonation, ozonation, mixed-media filtration, activated-carbon adsorption with thermal regeneration, final chlorination, and storage.

A portion of the reclaimed water is sold locally for golf course watering or for other purposes, and another portion is used to provide the injection water for an experimental groundwater barrier system to prevent intrusion of salt water from San Francisco Bay into the local freshwater aquifer. A long-term objective of research being conducted at the Reclamation Plant is to test the suitability of the reclaimed water, after injection and passage through the ground, as a potable supply.

The sampling locations for water evaluated in this particular study are shown in Fig. 1. They were as follows:

Sampling Location	Description
B	Activated-Sludge Effluent
C	Chlorinated Activated-Sludge Effluent
F	Recarbonation Effluent
G	Ozonated Effluent
H	Activated-Carbon Effluent
K	Reclamation Plant Effluent

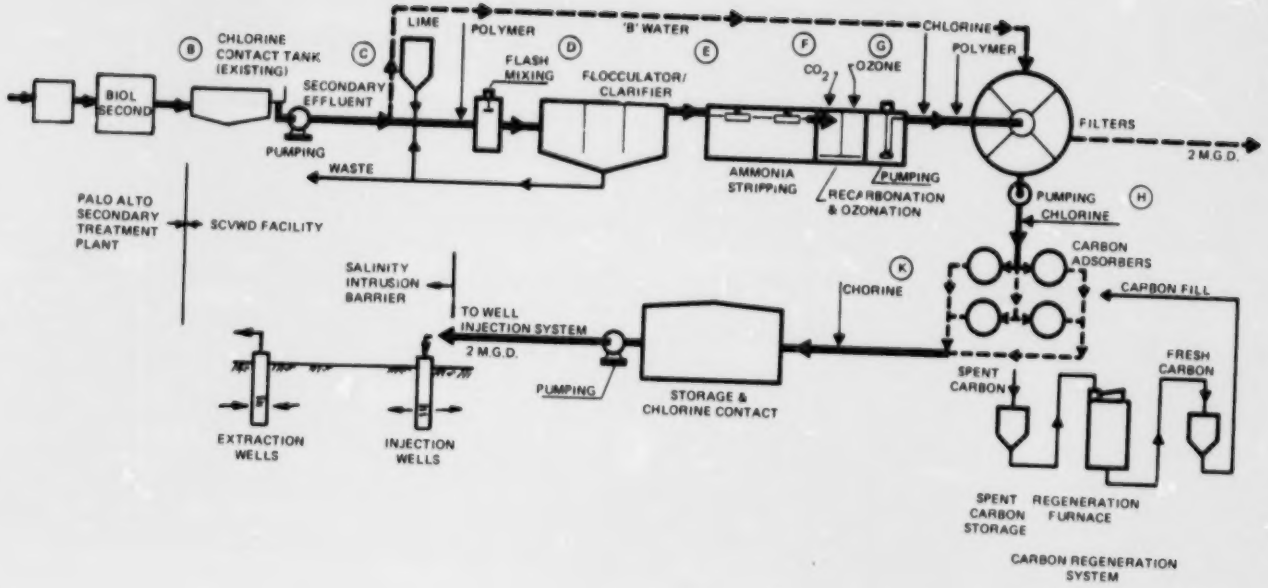


Figure 1. Flow diagram of Santa Clara Valley Water District Wastewater Reclamation and Injection/Extraction Well Facility at Palo Alto, California.

Table 1 is a summary of the operation of the reclamation plant during the portion of the study when the intensive chemical analysis program was conducted. The aerators and ozonater were in operation only during a portion of the study. This presented an opportunity to determine whether operational changes resulted in changes in mutagenic activity. When ozonation was discontinued after the 100th day of this study, the quality of samples at point G became similar to those at point F, except for filtration, which affected chemical quality very little. A comparison between points G and H indicated the effect of activated-carbon treatment, and comparisons between points B and C, and between points H and K allowed evaluations of the impact of chlorination.

Samples were also obtained of water which was injected into the ground and of water that had moved through the aquifer from the injection point to observation wells P-5, P-6, and P-7, located 10, 20, and 40 m, respectively, from the injection point. At the usual injection rate of 550 m^3/day, the flow time to each observation well, based upon tracer studies, was approximately 4, 16, and 67 hrs. The aquifer at the injection depth of 15 m was confined between clay layers and has a thickness of about 2 m.

TABLE 1. OPERATIONAL CHARACTERISTICS OF RECLAMATION PLANT DURING SAMPLING PERIOD
(Flow Rate: 0.044 m^3/s)

Date	Day of Study	Chlorine Additions, mg/l		Ozone Added (F) mg/l	pH		Ammonia Stripping Operated?	Granular Activated Carbon		
		Influent (B)	Effluent (H)		Lime Eff. (D)	Recarb. Eff. (F)		Empty-Bed Contact Time, min	Loading m^3/m^2-min	Total Flow Through GAC 10^3 m^3
3/30/79	54	12-16	4.8	5-6	9.5	7.5-8	No	35	0.13	19
4/10/79	65	8-12	4.3	5-6	9.5	7.5-8	No	35	0.13	61
4/21/79	76	11-15	3.6	5-6	9.5	7.5-8	No	35	0.13	102
4/26/79	81						No			
5/1/79	86	10-17	3.6	5-6	9.5	7.5-8	Yes	35	0.13	140
5/7/79	92				11		Yes			
5/10/79	95	10-17	3.6	5-6	11	7.5-8	Yes	35	0.13	174
5/14/79	99						Yes			
5/22/79	107	12-17	3.6	0	11	7.5-8	Yes	35	0.13	208
6/2/79	118	12-17	3.6	0	11	7.5-8	Yes	35	0.13	250
6/8/79	124						Yes			
6/13/79	129	12-17	3.6	0	11	7.5-8	Yes	35	0.13	292
6/19/79	135									
6/25/79	141	12-17	3.6	0	11	7.5-8	Yes	35	0.13	337
7/6/79	152	12-17	3.6	0	11	7.5-8	Yes	35	0.13	379

SECTION 5

SAMPLING AND ANALYTICAL PROCEDURES

SAMPLING

All analyses were conducted on grab samples collected by personnel from the Stanford University Water Quality Control Research Laboratory. Samples were collected in one- or five-gallon glass containers, one-liter glass containers, or in 120- or 60-ml hypovials, depending upon the particular analysis to be performed. All analyses for mutagenic activity were conducted at the Sanitary Engineering Research Laboratory, Richmond Field Station, University of California, Berkeley. Samples for the latter were collected in one-gallon amber glass bottles, packed with ice, and sent by bus to the Richmond Field Station, arriving there on the same day. In most cases samples were processed within two days of arrival at Richmond. However, on certain occasions, samples were refrigerated and stored up to ten days before processing.

Samples for chemical analyses were transported to the Stanford Laboratory and refrigerated prior to processing. Chemical analyses performed on these samples included those for group parameters as well as for specific organic compounds. In some cases, organic materials within samples were separated with resins similar to the procedures used for mutagenic analyses at the Richmond Laboratory, and the extracts obtained were subject to organic chemical analyses. Procedures followed for analyses of collective parameters, specific organic materials, and for mutagenic activity are described herein.

MUTAGENIC ACTIVITY

The organic material in one- or five-gallon samples for mutagenic analysis were first separated from the water by passage through organic resins for subsequent extraction and concentration. The concentrated organics were then subjected to mutagenic analysis.

Concentration of Organics

Organic material was extracted from the wastewater using a combination of XAD-2 and XAD-7 resins. The resins were obtained as 20/50 mesh from Mallinkrodt Chem., Inc. Approximately 0.7 kg of resin was washed in glass-distilled water followed by 95% ethyl alcohol before sequential washing in a soxhlet extractor with 95% ethyl alcohol, acetone, and ethyl ether. Extracts made with glass-distilled water were tested by the Ames procedure to confirm the absence of mutagenic activity in the acetone eluate of the cleaned resins.

A plug of glass wool was placed in the bottom of glass columns fitted with Teflon stopcocks, which were then packed with approximately 50 CC of XAD-2 followed by approximately 50 CC of XAD-7. The columns were rinsed with four liters of glass-distilled water prior to use. Before passing the wastewater through the column, sodium thiosulfate was added to the samples to remove any chlorine which might interfere with adsorption. The rate of flow through the columns was 60-80 ml per min.

After all the wastewater had passed through the column, and the residual water was blown out with nitrogen, glass-distilled acetone was used to elute the organic material from the resins. The eluate was collected in a beaker to which sodium sulfate was added to remove water, then filtered into a round-bottomed flask. Each extract was reduced to about 1 ml by vacuum evaporation at 32°C, then dried at 30°-32°C under a stream of dry nitrogen. The weight of the extract was determined, and then dissolved in dimethyl sulfoxide prior to storage at -70°C in Teflon-sealed vials.

Mutagenicity Testing

Mutagenicity testing was conducted using histidine-requiring mutants of Salmonella typhimurium. Standard tester strains were provided by Dr. Bruce Ames, University of California, Berkeley. Initial testing was conducted in all strains with and without S9 liver activation. Bacterial cultures were prepared fresh for each assay. Subsequent testing was carried out in strains TA98 and TA1535, both with activation, when it became apparent that the extracts were most active in these strains. Whereas the mutagenic activity in TA98 was enhanced with metabolic activation, activity in TA1535 did not require activation. However, the addition of liver fractions to plates with TA1535 appeared to detoxify the compounds responsible for the mutagenic activity in this strain.

Organic extracts were tested at three concentrations, ranging from 0.2-6.0 mg per plate. In addition, extracts were tested in duplicate whenever there was sufficient material. Revertant colonies were randomly picked and restreaked on histidine-free agar medium to confirm reversion to histidine-independence.

COLLECTIVE CHEMICAL PARAMETERS

In order to characterize the Palo Alto wastewater after various stages of treatment, analyses for the collective parameters of chemical oxygen demand (COD), total organic carbon (TOC), ultraviolet absorption (UV), and total organic halogen (TOX) were conducted. The latter analysis was subdivided into organic halogens that could be air-stripped or purged from samples (POX) and those that were non-purgeable (NPOX).

COD and TOC

COD was determined by the low-level dichromate reflux procedure outlined in the 14th Edition of Standard Methods (22).

TOC was determined with a Dohrmann-Envirotech DC-52 Organic Analyzer with an Oceanographics International ampule-crushing unit adapted to it. The analysis consisted of converting organic carbon to carbon dioxide by persulfate oxidation, and then quantification of the carbon dioxide by purging and flame-ionization detection.

Specifically for the TOC analysis, 0.5 to 5 ml of sample, 1 ml of a saturated potassium persulfate solution, 0.2 ml of 10% phosphoric acid solution, and deionized water were added to a glass ampule to give about 10 ml of final solution. This was purged for 7 to 9 min with purified oxygen; the ampules were sealed and autoclaved for 4 hrs at 130°C. The ampule was then cooled and crushed, and the carbon dioxide produced from organic carbon oxidation was purged with helium carrier gas into the flame-ionization detector. Results were calibrated against sucrose standards.

UV

UV absorption is sometimes used as a measure of the dissolved organic carbon content of water samples, and has been shown to exhibit a reasonable correlation with other collective parameters such as soluble TOC and COD (8). Samples for UV analysis were filtered through 0.45 μm membrane filters, and the absorption of ultraviolet light at a wavelength of 254 nm, using a 1-cm quartz cuvette, was recorded.

TOX, POX, NPOX

The procedures used for these analyses were developed at the Stanford University Water Quality Control Research Laboratory by Dr. Martin Jekel, and have been described in detail by Chow and Roberts (7). Volatile organics were first purged from a sample to determine the POX, and the remaining organics were then adsorbed onto activated carbon to determine NPOX. TOX is the sum of POX and NPOX. The organic halogen in both procedures was converted to inorganic halides by pyrolysis, and microcoulimetric titration was used to quantify the halide concentration. In the latter steps, a Dohrmann-Envirotech C-300 microcoulometer and a Dohrmann-Envirotech T-300-S titration cell were used.

Samples for organic halogen were collectd in 120-ml hypovials containing 1.0 ml of 0.1 N sodium thiosulfate solution to dispel residual chlorine. The filled hypovials were sealed with Teflon plugs and an aluminum outer seal, and stored at 4°C until analyzed.

For POX, a 50-ml sample was purged with a 1:1 by volume mixture of oxygen and argon, and the purged gases were passed through the pyrolysis and microcoulometric titration units.

For NPOX, 10 to 40 ml of the purged sample were acidified to pH 3 with 10% by volume sulfuric acid, and KNO_3 was added to give a 0.01 N concentration. Then 20 mg of 100/200 mesh powdered activated carbon from a 10 g/l stock solution was added, followed by 30 min stirring and filtration through a 0.45-μm, 25-mm dia. membrane filter. The process was repeated on the same sample and the filtered carbon was then rinsed with three 10-ml aliquots of

0.05 N KNO_3 under aspiration and then allowed to soak in the nitric acid solution for about 40 min to remove inorganic halides. The carbon was placed in a quartz or platinum boat, and pyrolyzed at 800°C in the above oxygen/argon mixture, which was passed to the microcoulometric titration unit. The standard deviation for the TOX procedure was found to be 30 µg/1.

SPECIFIC ORGANIC ANALYSES

Samples collected from the field were analyzed by three different gas chromatographic procedures in order to characterize some of the individual organic materials contained in the samples. The first two procedures, volatile organic analysis (VOA) and closed-loop stripping analysis (CLSA), were conducted directly on samples as obtained. These analyses measure constituents which are volatile and would thus be lost during evaporation of samples for mutagenic testing. It was felt to be important to analyze for these materials since some could be carcinogens. The third analytical procedure employed solvent extraction and methylation prior to gas chromatographic analysis, and was conducted on some of the concentrated extracts used for mutagenic analysis. This procedure, termed the methylated solvent extract analysis (MSEA), measures non-volatile compounds, including phenols and carboxylic acids.

VOA

Samples for VOA analysis were collected by filling 60-ml hypovials containing one ml of 0.1 N sodium thiosulfate and capped with Teflon seals. Analysis was by the pentane-extraction procedure (23). Organics were extracted with 1 ml of pentane containing internal standard (1,2-dibromoethane), and an aliquot of 5 µl was analyzed by gas chromatography on a 2-m packed column (10% squalene on chromosorb W/AW) using a linearized electron-capture detector. Results were integrated and computed by a System 1 integrator (Spectra Physics). Organics measured were mostly haloforms including chloroform, and various other halogenated one- and two-carbon organics. The detection limit was about 0.05 µg/1.

CLSA

Closed-loop stripping by the Grob procedure (24) allowed analysis for a broad range of volatile organics present down to the ng/1 range such as solvents, petroleum products, and chlorobenzenes. However, several of the organics determined by VOA are not measured quantitatively by this method. Thus, VOA and CLSA are complementary procedures. One-liter bottles (solvent cleaned) were filled and refrigerated until analyzed. Organics in 250-1000 ml of sample were recovered by recirculation of a small volume of air through the sample and over an activated charcoal filter for two hours. The filter was then extracted with 20 µl of CS_2, approximately 13 µl of which was recovered. An aliquot of 1.5 µl was used for high-resolution gas chromatographic (GC) analysis (Carlo Erba), using a glass capillary column (50-m UCON HB, Jaeggi Laboratory for GC, Trogen, Switzerland), a Grob-type injector (Brechbühler AG, Urdof, Switzerland), and flame-ionization detection. Chromatograms were analyzed with a Sigma 10 reporting integrator. For mass spectrometric (MS)

identification (Finnegan 4000), a 3-µl aliquot was used. Monochlorinated normal alkanes (1-Cl-C_8H_{17}, 1-Cl-$Cl_{12}H_{25}$, and 1-Cl-$C_{16}H_{33}$) were added to samples as internal standards prior to air stripping. The detection limit for individual compounds was about 0.01 µg/l.

MSEA

Organic materials for this analysis were concentrated on XAD resins in a way similar to that done at the University of California Richmond Laboratory (see method for concentration of organics for mutagenic activity). Aliquots of these extracts were also sent to the Richmond Laboratory for mutagenic testing.

Cleaned and combined XAD-2 and XAD-7 resins were placed in columns and eluted with four bed volumes of acetone at approximately 5 ml/min. The eluate was discarded and the procedure was repeated; this second eluate was saved to serve as a resin blank. The column was then washed with acidified (pH 1) or neutral (pH 6 to 7) distilled water, depending upon pH of the sample to be concentrated, and then about 3.8 liters of neutral or acidified (2 ml conc. sulfuric acid per liter) was passed through the column at about 18 ml/min. The organics were extracted from the column with 280 ml of glass-distilled acetone and directed onto 30 g of anhydrous sodium sulfate in a one-liter round-bottom flask to remove water.

Acetone extracts were stored at -15°C in the dark for up to four days, during which time some of the organics precipitated and would not return to solution upon warming to room temperature. The extracts were passed again through anhydrous sodium sulfate and the acetone was evaporated at 1/2 bar pressure and 35°C, leaving a few ml of water behind, which was removed by heating to 60°C under full aspirator vacuum. The residue was dissolved into methanol, 3/4 of which was placed in a tared 7-ml screw-cap vial sealed with a Teflon-silicon disc (Pierce Chemical Co., Rockford, Illinois), evaporated to dryness under a stream of nitrogen, and the organics remaining were weighed before being redissolved in 1 ml of dimethylsulfoxide (DMSO) for mutagenic testing at the Richmond Laboratory.

The remainder of the methanol extract was used for gas chromatographic analysis. It was placed in a second 7-ml vial and methylated by mixing with 4 to 5 ml of an etheral-alcoholic diazomethane solution and left to sit overnight with the vial sealed. The sample was then concentrated to 100 µl under a stream of nitrogen, 6 µl of a 2.5 mg/ml solution of 3,6-dimethylphenanthrene (internal standard) in acetone was added, and the sample was diluted to 200 µl with ethyl ether. Three µl was used for gas chromatographic/mass spectrometric analysis (Finnegan 4000) using splitless injection onto a 50-m SE-54 0.33-mm I.D. glass capillary column, helium carrier gas at 1-bar pressure, and an initial temperature of 80°C for 4 min followed by temperature increase at 3°C/min up to 250°C, which was held for 10 min. Many compounds could be identified from the mass spectra generated, but many others could not be in the time available for this study.

SECTION 6

IDENTIFICATION AND REMOVAL OF CHEMICAL CONSTITUENTS

Grab samples were collected at various sampling locations at the Palo Alto Reclamation Plant in the afternoon at one-half week to weekly intervals during the ten-week study period. At each time of collection, samples were obtained for measurement of collective chemical parameters, mutagenic activity, and individual organic chemicals as measured by VOA and CLSA.

The collective parameters of COD, TOC, UV, TOX, POX, and NPOX were measured in order to determine whether any correlation could be made between the concentration of these materials and mutagenic activity. In addition, some samples that were concentrated for mutagenic activity were also subject to analysis by MSEA in order to determine the nature of the organic materials in these concentrates. Of course, gas chromatographic procedures can measure only a small fraction of the total organic materials in the samples, but even so, the limited identification was felt to be desirable.

The results of the mutagenic activity measurements and MSEA are given in the following section. In this section, the concentration of collective constituents and individual volatile organic materials are summarized, together with the efficiency of the treatment plant in their removal.

The detailed results of the chemical analyses are given in the Appendix, and only a brief summary is provided in this section. Listed in Table 2 is a summary of the average concentrations of collective parameters and volatile organic materials found during this study. The average concentrations shown are geometric means, determined by the procedures reported previously (4). The overall percent removals indicated are based upon the difference between the influent concentrations to the reclamation plant and the effluent concentrations. The removals of individual collective parameters and individual organic materials varied markedly from about zero for carbon tetrachloride and heptaldehyde, through about 60 to 80% for collective parameters such as COD, TOC, UV, and TOX, to greater than 99% for the chlorinated ethanes and ethylenes.

The concentrations of some materials, such as the trihalomethanes (chloroform and the subsequent three halogenated methanes), both decreased and increased during treatment. The increases generally resulted from the formation of trihalomethanes following the addition of chlorine for disinfection. A similar behavior was exhibited for the halogenated organics as measured by TOX, POX, and NPOX.

TABLE 2. SUMMARY OF GEOMETRICAL MEAN CONCENTRATIONS AND PERCENT REMOVALS FOR COLLECTIVE PARAMETERS AND INDIVIDUAL VOLATILE COMPOUNDS

Analysis	Units†	Effluent* (B)	(C)	(F)#	(G)#	(H)	(K)	Overall Removal %
COD	mg/l	38.1	41.5	23.9	-	10.7	7.8	79
TOC	mg/l	10.9	10.4	6.9	8	2.5	2.2	80
UV	cm^{-1}	0.15	0.18	0.16	0.12	0.03	0.05	67
TOX		192	297	166	205	71	65	66
POX		135	120	7	45	12	10	93
NPOX		117	168	159	150	53	46	61
Chloroform		13.0	16.2	1.2	4.8	2.1	2.3	82
Bromodichloro-methane		0.23	1.6	0.12	1.2	0.04	0.15	35
Dibromochloro-methane		0.13	0.50	0.09	0.36	<0.05	0.04	69
Tribromomethane		<0.05	0.10	0.03	<0.05	<0.05	<0.05	?
Carbon tetra-chloride		0.10	0.20	<0.05	<0.05	0.02	0.10	0
1,1,1-trichloro-ethane		65	65	0.7	3.3	0.4	0.4	99.4
Trichloroethylene		25	27	0.34	3.6	0.06	0.01	99.96
Tetrachloro-ethylene		44	47	0.4	3.6	0.17	0.16	99.6
p-Xylene		0.06	0.29	0.04	0.06	<0.01	<0.01	>83
m-Xylene		0.20	1.1	0.09	0.39	<0.01	0.02	90
Ethylbenzene		0.03	0.07	0.05	0.02	<0.01	<0.01	>67
Chlorobenzene		0.29	0.83	0.25	0.93	0.14	0.19	34
o-Dichlorobenzene		2.7	3.8	0.17	0.95	0.02	0.02	99
m-Dichlorobenzene		3.6	4.7	0.34	1.3	0.03	0.07	98
p-Dichlorobenzene		5.4	6.7	0.61	3.4	0.04	0.12	98
1,2,4-trichloro-benzene		11.3	13.0	1.3	2.1	0.04	0.28	98
Naphthalene		3.3	3.5	1.0	1.1	0.04	0.25	92
Heptaldehyde		<0.01	0.51	0.08	3.8	0.05	0.12	0
Pentylcyanide		0.06	0.33	0.06	0.71	<0.01	<0.01	83
Hexylcyanide		0.004	0.47	0.09	1.3	<0.01	<0.01	?
Heptylcyanide		0.04	0.28	0.06	0.37	<0.01	<0.01	>75
Octylcyanide		0.10	1.0	0.11	1.5	<0.01	<0.01	>90
Nonylcyanide		<0.01	0.26	0.05	0.5	<0.01	<0.01	?

*(B) = Secondary Effluent; (C) = Chlorinated Secondary Effluent; (F) = Recarbonated Effluent; (G) = Ozonated Effluent; (H) = Activated-Carbon Effluent; (K) = Reclamation Plant Effluent.

†µg/l unless otherwise shown.

#For the first 100 days, samples were taken at point F, and after that at point G, thus differences in averages which may be shown do not reflect the effects of consecutive treatment steps.

For most of the individual organic materials measured, overall percent removals were greater than for overall organics as measured by the collective parameters. This was largely the result of the volatile nature of the former materials. They were removed quite efficiently by the air-stripping process. This process was used only during the last six weeks of this study, and for this reason the averaged data in Table 2 may be somewhat misleading. In order to illustrate the effectiveness of the stripping process, the data for the last five weeks of operation (days 107 to 152) were analyzed, and the percent removals as indicated by paired data were determined. The results are summarized in Table 3. Removals by lime treatment and air-stripping were based upon a comparison of paired data from sampling locations C and F. Removals by ozonation, filtration, and activated-carbon adsorption were determined by a comparison of paired data from sampling points G and H.

TABLE 3. EFFECTIVENESS IN ORGANIC REMOVALS OF GROUPS OF PROCESSES INCLUSIVE OF STRIPPING AND ACTIVATED CARBON

	Lime Treatment, Stripping, and Recarbonation			Filtration and Act.-Carbon Adsorption		
Analysis	Inf. Conc. (C)*	Eff. Conc. (F)*	Removal %	Inf. Conc. (F)*	Eff. Conc. (H)*	Removal %
COD	42	24	43	24	11	54
TOC	11	7	38	6.8	2.8	59
UV	0.18	0.16	16	0.15	0.04	74
TOX	329	167	49	170	114	33
POX	152	7	95	6	10	-53
NPOX	169	160	5	163	104	36
Chloroform	18.1	1.4	92			
Bromodichloromethane	1.91	0.10	95			
Dibromochloromethane	0.50	0.08	83			
1,1,1-trichloroethane	105	1.0	99	0.57	0.56	2
Trichloroethylene	38	0.4	99	0.38	0.04	88
Tetrachloroethylene	58	0.4	99	0.33	0.16	50
Chlorobenzene	0.65	0.28	57	0.21	0.14	32
o-Dichlorobenzene	3.1	0.19	94	0.15	0.01	92
m-Dichlorobenzene	5.1	0.40	92	0.28	0.02	93
p-Dichlorobenzene	8.3	0.75	91	0.48	0.02	95
1,2,4-trichlorobenzene	14.2	1.1	92	1.5	0.04	97
Naphthalene	3.0	0.8	75	1.3	0.08	94

*(C) = Chlorinated Secondary Effluent; (F) = Recarbonated Effluent; (H) = Activated-Carbon Effluent.

The combination of processes involved between each of these locations makes it difficult to draw firm conclusions about which process was most effective, but some generalizations are possible based upon previous studies of advanced wastewater treatment (5). For example, chemical treatment removes suspended materials and also a portion of the higher molecular weight dissolved organics (6). This is the main process effective in removal of COD, TOC, UV, and NPOX. The reduction in UV absorbance is not as high as removals of COD and TOC, probably because UV measures only dissolved materials, while COD and TOC measure suspended materials as well. The low removal of NPOX might be explained on a similar basis. On the other hand, the high removals of the individual volatile organics and POX took place primarily through air-stripping.

In the combination of processes which included activated-carbon adsorption, this process was no doubt the one responsible for most removals obtained. It is interesting to note that while activated carbon was relatively efficient in removing COD, TOC, and UV, it was even better for the removal of the chlorinated benzenes. Data are not shown for removal of the trihalomethanes during activated-carbon adsorption, but during the last 5 weeks, removal was essentially zero. However, removal was reasonably good during the first five weeks of the study when the activated carbon was new. Thus, for the last five weeks of operation it lost its capacity to remove the trihalomethanes.

In summary, the Palo Alto Reclamation Plant performed well in the removal of many organic materials. This removal occurred through lime treatment and stripping, as well as through activated-carbon adsorption. However, some materials such as the trihalomethanes and TOX were also formed during treatment through chlorination. Even though removal of many materials was quite adequate, the effluent from the Reclamation Plant still contained many identifiable as well as unknown organic materials. It is of importance to determine whether there is a health risk associated with the presence of these materials in drinking water and so further studies are desirable. The evaluation of mutagenic activity given in the following section was conducted to aid in this evaluation.

SECTION 7

MUTAGENIC ACTIVITY OF SECONDARY EFFLUENT AND RECLAIMED WASTEWATERS

INTRODUCTION

Over the period of this study, samples were collected for chemical analyses and for determination of mutagenic activity at various sampling locations at the Palo Alto Reclamation Plant, and from monitoring wells near the injection well. The results of analyses for collective chemical parameters and individual volatile organic compounds are given in the previous section. This section contains results of the mutagenic activity measurements as well as a summary of individual organic compounds identified in some of the sample concentrates used for mutagenic analysis. There were three specific studies of mutagenic activity. The first was of activity in samples taken from various sampling locations at the Reclamation Plant itself, the second was of samples taken from monitoring wells following injection of the reclaimed water, and the third was for a special study on the effect of disinfection on production of materials with mutagenic activity.

MUTAGENIC ACTIVITY OF RECLAMATION PLANT SAMPLES

Samples received at the Richmond Field Station were concentrated by procedures outlined in Section 5 of this report. The recovery of XAD-acetone-extractable material from the samples taken from various sampling locations of the Reclamation Plant are summarized in Table 4. An estimate was made of the percent of total organic material in the original sample that the XAD-acetone-extractable material represented. The concentration of organic material in the sample was estimated to be twice the TOC concentration (Appendix Tables A-1 through A-6). On this basis, the average recovery of organics equalled 11.7% (s.d. 8.7%). This represents a relatively small percentage of the total organic concentration. The mutagenic characteristics of the remaining fraction could not be determined by the procedures used.

Table 5 contains a summary of the mutagenic activity and the concentrations of TOX, POX, and NPOX of the sample for comparison. The values for specific activity (revertants/mg) were derived from the slope of the linear position of the dose-response curve of each extract (Fig. 2). The values for revertants per liter were calculated from the specific activity. Mutagenic activity is defined as the net number of revertant colonies divided by the spontaneous revertants. No value was determined when extracts did not exhibit a dose-response to the test bacteria or when the number of revertants at the highest dose tested was less than double the spontaneous count (NDR). The

TABLE 4. RECOVERY* OF ORGANIC MATERIAL FROM 3.7 LITERS OF WASTEWATER BY XAD-ACETONE EXTRACTION

Day of Sampling	Primary Eff. (A)		Act.-Sludge Aff. (B)		Chlor. Act.-Sludge Eff. (C)		Recarbon. Eff. (F)		Ozonated Eff. (G)		Act.-Carbon Eff. (H)		Reclam. Plant Eff. (K)	
	mg recov.	% recov.	mg recov.	% recov.	mg recov.	% recov.	mg recov.	% recov.	mg recov.	% recov.	mg recov.	% recov.	mg recov.	% recov.
1	19.4		7.8		6.5						4.6			
11	35.5†		14.0†		12.7						5.8			
54			5.0		13.0				8.2		3.9	21.0	3.7	21.7
65			4.9		10.1				15.1		9.3		7.1	
76			8.7	10.7	13.7	16.8			10.5		2.6			
86			7.2	8.5	7.2	13.0			5.3	7.2	8.0	25.7	1.7	8.2
107			5.5		5.8	6.3	4.8	8.6			3.8	36.7	3.3	21.2
118			6.9	8.9	7.8	11.7	8.5	14.4					3.3	27.9
129			7.1	8.7	5.8	5.8	3.3	6.4			1.1	4.1	1.3	5.9
141			6.1	7.9	4.9	5.8	2.8	9.5			1.4	17.2	und‡	
152			6.2	7.6	4.9	7.4	2.7	4.3			0.62	1.8	0.31	1.3
Avg. mg recov.	43±33		8.3±6.0		8.4±3.4		4.4±2.4		9.8±4.1		4.1±2.9		3.0±2.2	
Avg. % recov.				8.7 ± 1.1		9.5 ± 4.3		8.6 ± 3.8		7.2		17.8 ± 13.2		14.4 ± 10.6

*Percent recovery estimated from 100[mg recov./3.74(2)(TOC)], overall for 31 samples = 11.7 ± 8.2%.

†Sample volume = 2 liters.

‡Recovery undetermined, sample dried out in rotary evaporator.

TABLE 5. SUMMARY OF MUTAGENIC ACTIVITY AND ORGANIC HALOGEN CONCENTRATION OF WASTEWATER SAMPLES

		TA98				TA1535				Organic Halogen µg/l		
Day	Sample Loc.	Rev/mg[a]	Act[b]	Rev/l[c]	Act/l	Rev/mg	Act	Rev/l	Act/l	TOX[d]	POX[e]	NPOX[f]
1	A	67	2.2	1086	36.2	NDR[g]	-	-	-			
	B	50	1.6	327	10.9	NDR	-	-	-			
	C	110	3.7	713	23.8	50	4.5	324	29.4			
	H	125	4.2	650	18 0	NDR	-	-	-			
11	A	32	1.5	1124	51.1	NDR	-	-	-			
	B	17	0.8	235	10.6	NDR	-	-	-			
	C	15	0.68	210	9.5	30	3.8	420	24.7			
	H	7	0.32	41	1.8	NDR	-	-	-			
54		no data -- all plates contaminated										
65	B	30	1.5	148	7.4	NT	-	-	-	149	61	88
	C	25	1.1	252	12.6	20	2.5	202	25.2	250	78	172
	G	14	0.64	212	9.6	NDR	-	-	-	181	38	143
	H	NDR	-	-	-	NDR	-	-	-	27	15	12
	K	NDR	-	-	-	NDR	-	-	-	30	21	9
76	B	89	3.4	235	9.0	NT	-	-	-	-	-	-
	C	27	1.03	371	14.2	105	13.2	1438	179.8	221	70	151
	G	30	1.4	315	12.1	11	1.4	116	14.4	205	93	112
	H	6	0.27	15	0.70	NDR	-	-	-	47	16	31
86	B	65	2.5	469	18.0	NDR	-	-	-	177	72	105
	C	54	2.1	390	15.0	295	36.8	2128	266.1	282	79	203
	G	48	1.8	259	9.7	11	1.4	58	7.2	244	36	208
	H	5	0.19	40	1.5	NDR	-	-	-	92	18	74
	K	190	7.3	324	12.4	NDR	-	-	-	141	7	134
107	B	44	1.5	242	8.4	NDR	-	-	-	336	180	156
	C	62	2.1	358	12.4	60	10.0	347	57.8	297	92	205
	F	35	1.2	168	5.7	23	3.8	110	18.4	258	9	249
	H	7	0.24	27	0.93	4	0.67	15.3	2.6	243	15	228
	K	18	0.62	60	2.1	NDR	-	-	-	226	10	216
118	B	59	2.0	407	14.0	NDR	-	-	-	238	111	127
	C	48	1.7	374	12.9	7	1.2	54	9.1	266	100	166
	F	39	1.3	330	11.4	7	1.2	59	9.9	154	10	144
	K	11	0.38	36	1.2	4	0.67	13	2.2	61	7	54
129	B	34	0.74	241	5.2					193	76	117
	C	40	0.87	232	5.0					283	150	133
	F	60	1.3	198	4.3					134	3	131
	H	NDR	-	-	-					97	11	86
	K	NDR	-	-	-					60	8	52

TABLE 5 cont.

TABLE 5 cont.

Day	Sample Loc.	TA98 Rev/mg[a]	TA98 Act[b]	TA98 Rev/1[c]	TA98 Act/1	TA1535 Rev/mg	TA1535 Act	TA1535 Rev/1	TA1535 Act/1	Organic Halogen µg/1 TOX[d]	POX[e]	NPOX[f]
141	B	50	1.1	305	6.6					438	312	126
	C	26	0.56	127	28					404	237	167
	F	50	1.3	168	3.6					147	8	139
	H	NDR	-	-	-					108	7	101
	K	200	4.3	280	6.1					77	12	65
152	B	45	2.1	279	13.3	NDR	-	-	-	446	310	136
	C	68	3.2	333	15.9	NDR	-	-	-	414	238	176
	F	115	5.5	310	14.8	10	1.2	28	3.5	161	7	154
	H	16	0.76	10	0.47	NDR	-	-	-	66	7	59
	K	106	5.0	33	1.6	NDR	-	-	-	84	8	76

[a]Rev/mg - slope of dose response counts.

[b]Act - activity, net revertants/spontaneous revertants.

[c]Rev/1 - revertant/liter, calculated from rev/mg.

[d]TOX - total organic halogen as Cl (µg/1).

[e]POX - purgeable organic halogen (µg/1).

[f]NPOX - non-purgeable organic halogen (µg/1).

[g]NDR - no dose response.

values for strain TA1535 were based on plates which included the addition of S9 liver fraction because, as mentioned previously, of the toxicity of some extracts to this strain. Activation, however, was not necessary for the extracts to be mutagenic in strain TA1535.

It appears that two types of activity occurred during the treatment process. On all of the sampling dates mutagenically active compounds were found in the secondary effluent entering the reclamation facility. These mutagens are of the frameshift type and required metabolic activation before inducing mutagenesis in strain TA98. The compounds responsible for this activity were perhaps introduced into the plant by various industries in the area. In addition to the mutagens entering the plant, new mutagens were formed following chlorination of the secondary effluent. These compounds are base-pair substitution mutagens and did not require metabolic activity to induce mutagenesis in strain TA1535.

The movement of the frameshift mutagens through the treatment plant during the ten sampling periods as determined by strain TA98 is summarized in Fig. 3a-j, the data for which were taken from Table 5. The mean mutagenic activity of the secondary effluent without chlorination was 10.3 per liter

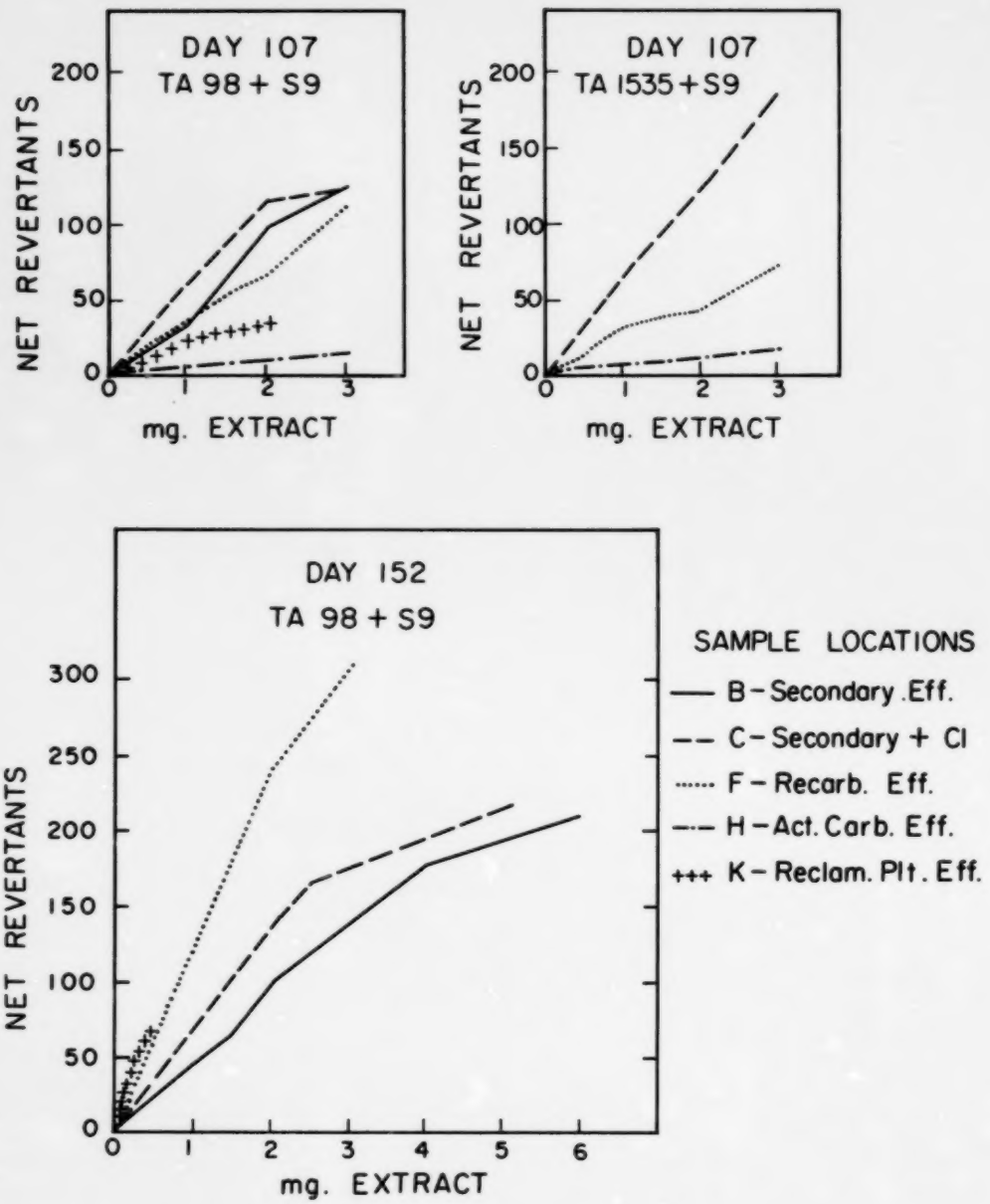


Figure 2. Examples of dose-response curves of extracts to test bacteria.

(s.d. 3.9%). Following chlorination, this mutagenic activity tended to remain the same or increase, rather than decrease, and had a mean value of 12.3 per liter (s.d. 5.8%). Subsequent processes of ozonation and stripping were also ineffective in removing these mutagens. However, the following process of activated-carbon treatment consistently removed the mutagenically active material, except on one occasion (Day 1, Fig. 3a), when the activity of influent to the carbon was 13.8 and the effluent was 18.

The next step in the process after activated-carbon treatment was chlorination. This tended to increase the frameshift mutagenic activity. This was most noticeable on Day 86 (Fig. 3e) and Day 141 (Fig. 3j), when the mutagenic activity of the finished water was 12.4 and 6.1 per liter, respectively. The precursors of these compounds might have been formed during ozonation or activated-carbon treatment, or they might have been present in the reclamation plant influent and unaffected by the initial chlorination step. It is also interesting to note that the increase in activity of the chlorinated carbon-treated effluent on Day 86 was accompanied by a significant increase in the NPOX (Table 5), but no such increase in NPOX was noted on Day 141.

While chlorination of secondary effluent did not cause a statistically significant rise in frameshift mutagens, it did result in a large increase in base-pair substitution mutagenic activity as measured by strain TA1535. This is illustrated in Figs. 4a-h. No mutagenic activity was detected in secondary effluents with TA1535, whereas most of the chlorinated secondary effluent extracts induced mutagenesis in this strain. This activity varied greatly from 9.1 to 266 per liter. The variation may be due to a number of factors, including differences in the concentration and types of precursor compounds present, and differences in operating conditions at the reclamation plant over the period of the study. Another source of variation may be in the differing amounts of time between sampling and sample processing. In contrast to the frameshift mutagens, ozonation was much more effective in removing the base-pair substitution compounds, and chlorination of the carbon effluent did not produce additional compounds that were active to TA1535.

During chlorination of secondary effluent, the concentrations of NPOX and the mutagenic activity to strain TA1535 always increased. Thus, a cause-and-effect relationship may be suggested. However, following ozonation the decrease in activity in TA1535 was not generally accompanied by a similar decrease in the NPOX concentration. Thus, a close correlation between these two parameters is not indicated.

EFFECT OF DISINFECTION ON MUTAGENIC ACTIVITY

A comparison of samples of activated-sludge effluent (sample location B) with chlorinated activated-sludge effluent (sample location C) in Table 5 suggests that following chlorination, the mutagenic activity generally increased. This was especially true for strain TA1535. In order to confirm this, a special study was conducted on August 8, 1979 (Day 185). Unchlorinated activated-sludge effluent was obtained and taken to the Stanford Water Quality Control Research Laboratory. The sample was divided into six parts. Two portions were left unchlorinated, two portions were each chlorinated with

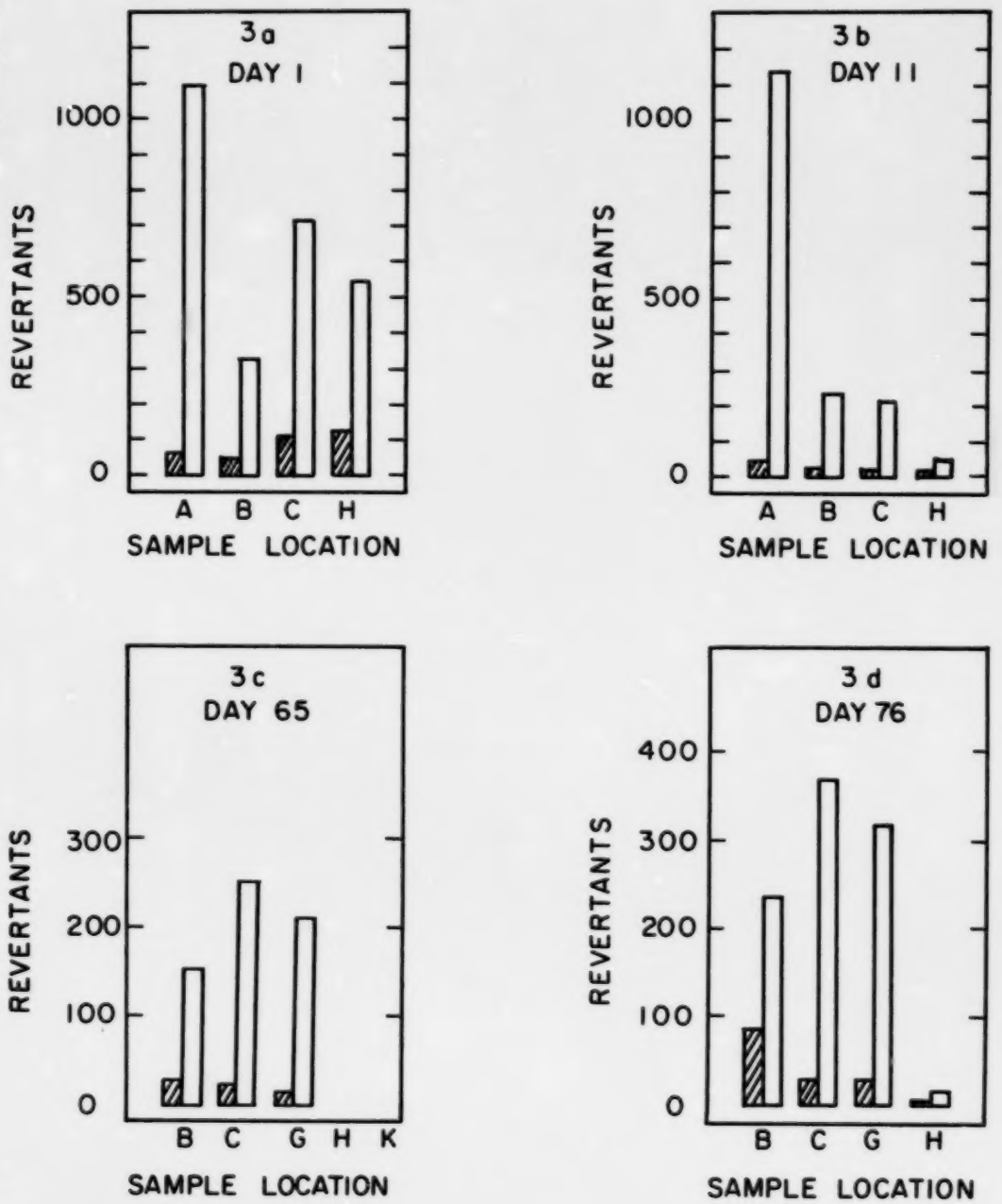
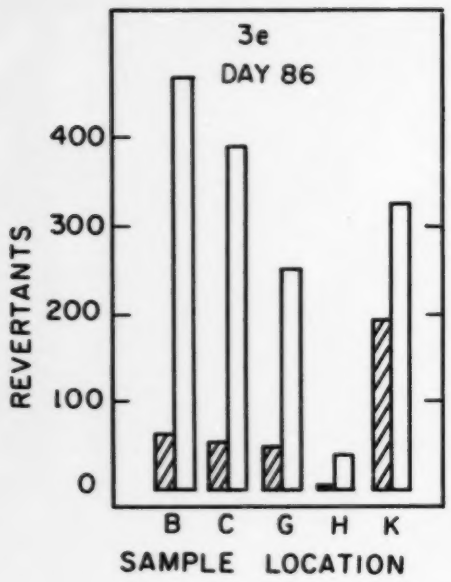
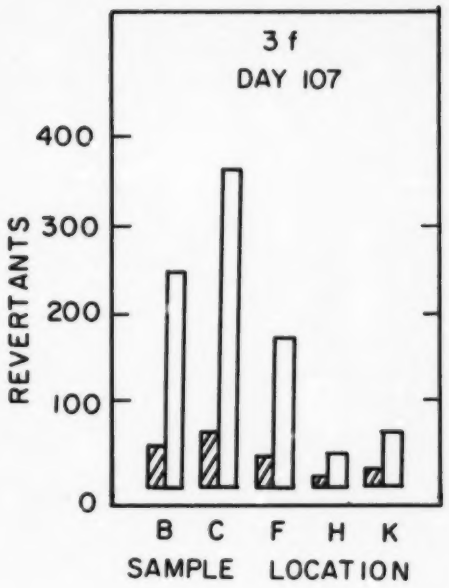
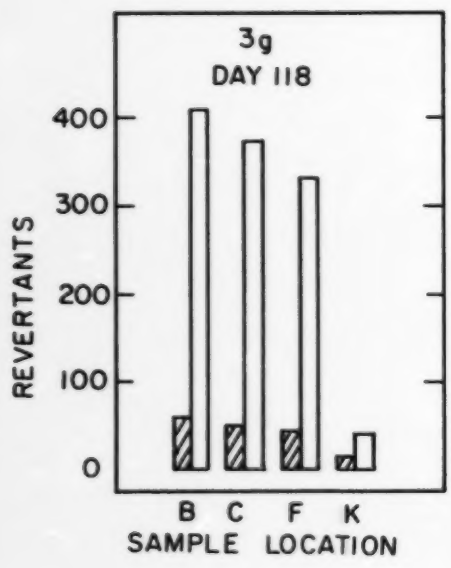


Figure 3. Mutagenic activity of wastewater effluents to frameshift mutagen TA98 with S9 liver activation - (cont.).







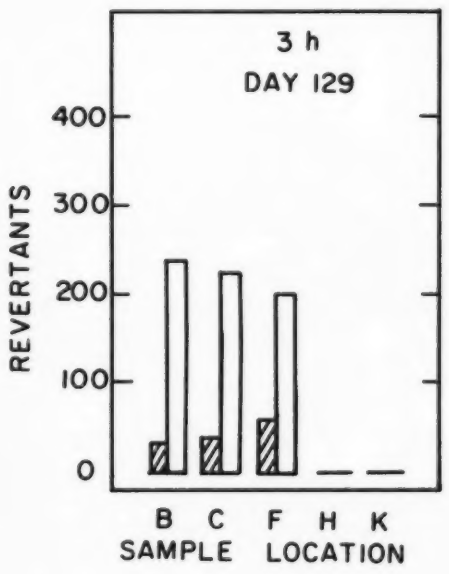
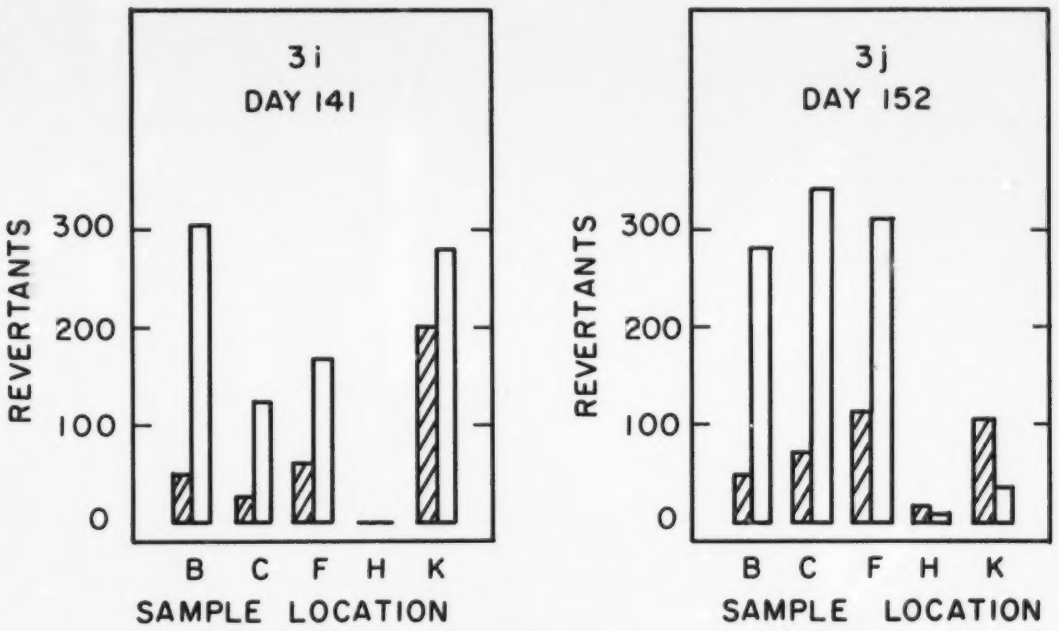


Figure 3 continued.



SAMPLE LOCATIONS

A – Primary Eff.

B – Secondary Eff.

C – Secondary + Cl_2

F – Recarb. Eff.

G – Ozonated Eff.

H – Act. Carb. Eff.

K – Reclam. Plt. Eff.

Number revertants / Liter

Number revertants / mg

Figure 3 continued.

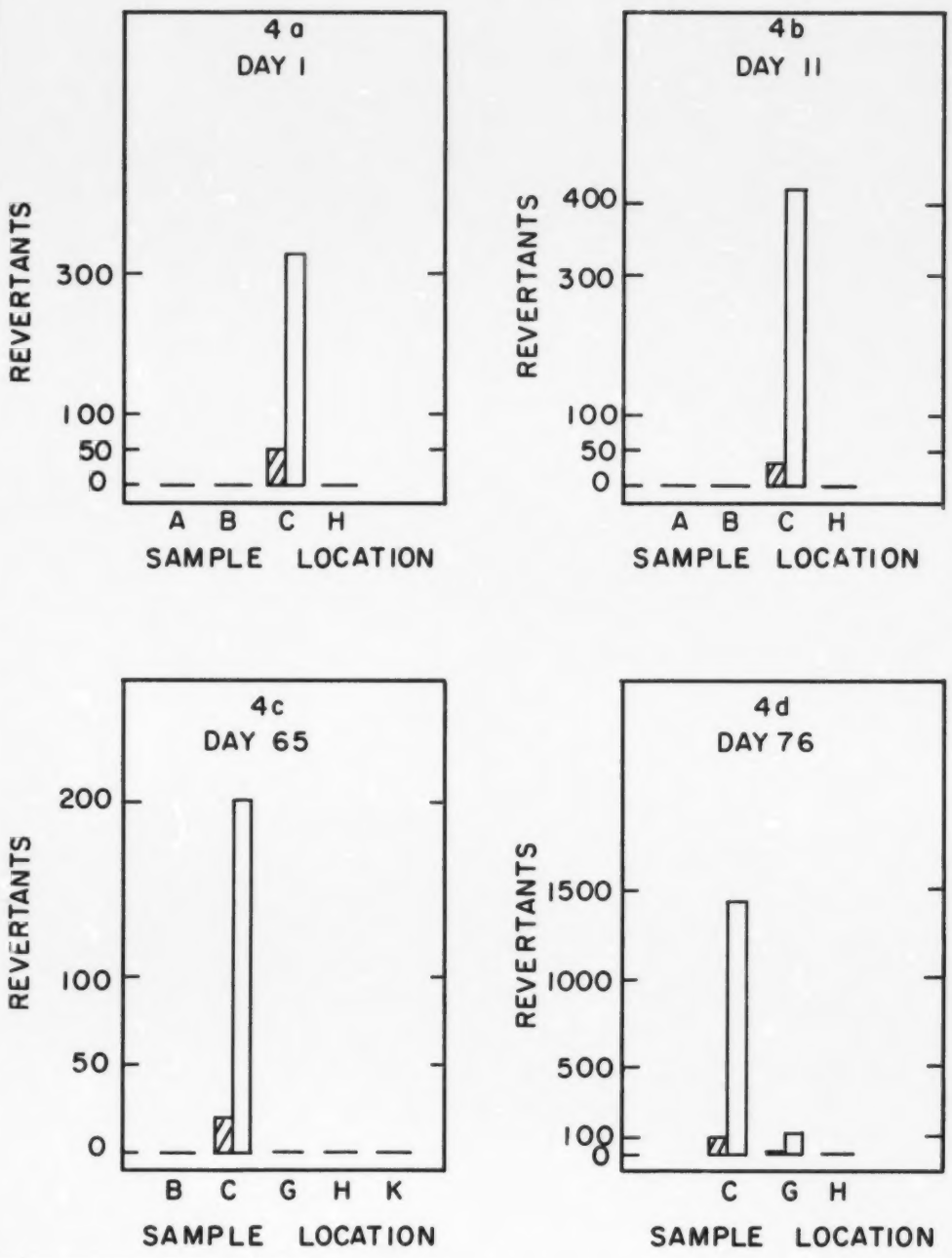


Figure 4. Mutagenic activity of wastewater effluents to base-pair substitution mutagen TA1535 with S9 liver activation - (cont).

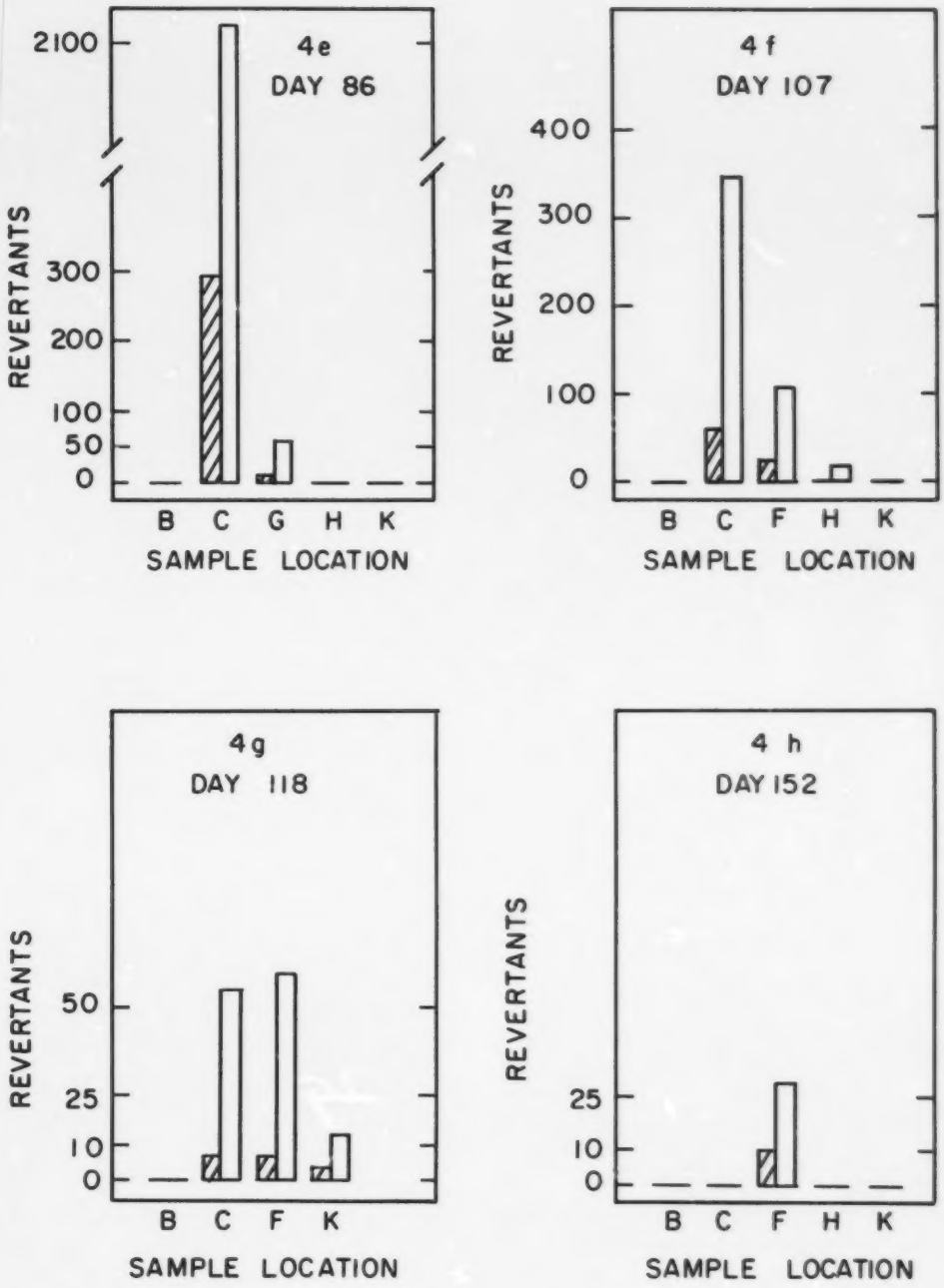


Figure 4 continued.

20 mg/l as chlorine from a chlorine solution, and two portions were treated with 20 mg/l of chlorine dioxide from a chlorine-free solution prepared as described previously (7). The latter was used in order to evaluate the effect of an alternate disinfectant. The samples were kept undisturbed for 24 hrs at room temperature, and then aliquots of each portion were sent to the Richmond Laboratory for mutagenic analysis, and the remainder was analyzed at Stanford for residual chlorine, chlorides, POX and NPOX, and TOC.

The results of the chemical analyses are summarized in Table 6. At the end of the 24-hr period, both chlorine and chlorine dioxide residuals remained

TABLE 6. COMPARISON OF ORGANIC HALOGENS IN SECONDARY EFFLUENT DISINFECTED WITH CHLORINE AND CHLORINE DIOXIDE

Sample	Residual (24 hr) mg/l	Chloride (24 hr) mg/l-Cl	POX* µmol/l-x	NPOX† µmol/l-x	TOC mg/l-C
(1) Cl_2	9.5	193	3.5	> 7.0	12
	10.5	195	3.7		
	as Cl	194			
(2) Cl_2	10.3	194	3.4	> 7.0	12
	10.3	195	2.2	> 7.0	
	as Cl	193			
(3) ClO_2	2.3	185	3.5	3.4	12.5
	2.3	185	3.2	3.5	
	as ClO_2	184			
(4) ClO_2	1.8	185	3.1	3.8	12.5
	1.3	185	3.2	2.9	
	as ClO_2	184			
(5) Control	-	186	3.3		
		184	3.7	2.7	11.5
		184			
(6) Control	-	184	3.6	3.0	
		184	2.9	3.0	11

*Purgeable Organic Halogen (9-min purge at ambient temperature).

†Non-Purgeable Organic Halogen (carbon adsorbable)

Conditions	Water Characteristics
Water: Palo Alto secondary eff.	COD: 51 mg/l; TOC: 12 mg/l
Dose: 20 mg/l as Cl_2	NH_3-N: 25 mg/l-N
20 mg/l as ClO_2	TKN: 27 mg/l-N
Temperature: 25°C	pH: 6.8
Contact Time: 24 hrs	Alk: 212 mg/l $CaCO_3$
	Susp. Sol.: 15 mg/l

in the samples to which these materials were added. There was little difference between the concentration of purgeable organic halogens present in the different samples, but in those receiving chlorine, the concentration of nonpurgeable organic halides was at least twice that in the other samples. Thus, chlorine did react with the organic materials present.

The samples for mutagenic activity testing were analyzed with five different strains, TA98, TA100, TA1535, TA1537, and TA1538. The results for the three strains which indicated positive mutagenic activity are shown in Fig. 5. With TA98 plus S9 liver fraction, there was little, if any, difference in mutagenic activity between the chlorinated and unchlorinated activated-sludge effluents. One of the duplicates with chlorine dioxide showed no difference from the other samples, while the other had a somewhat lower activity. Thus, the results with chlorine dioxide are not conclusive.

For TA1535, the response was similar to that observed with the field samples, the chlorinated samples were the only ones showing significant mutagenic activity. The activity was not high, but nevertheless was in the same order of magnitude generally found for the reclamation plant samples. Mutagenic activity was also found with the frameshift strain, TA1538 with S9 liver fraction. However, with this strain, chlorination did not seem to increase activity, but chlorine dioxide did appear to decrease the activity.

These results illustrate that the chlorination of Palo Alto activated-sludge effluent does increase the mutagenic activity to TA1535, but apparently not to the extent of other strains tested. Also, this increase in activity did not occur when an alternate disinfectant, chlorine dioxide, was used.

ORGANIC COMPOUNDS IN XAD-ACETONE EXTRACTS

Some samples obtained by passing wastewater through the combined XAD-2 and XAD-7 resins and extracting with acetone were analyzed by GC/MS in order to identify some of the organic compounds present. These particular samples were prepared in the Stanford Water Quality Control Research Laboratory using essentially the same concentration techniques as at the University of California, Richmond Laboratory, for mutagenic analysis. The procedures followed in preparing the extracts and conducting the analysis are outlined in Section 5 under Specific Organic Analyses (MSEA). Since the concentration procedures involved sample evaporation, all volatile compounds were lost. After evaporation, the remaining organic materials were dissolved in methanol, methylated in order to make carboxylic acids and phenols volatile for analysis by gas chromatography, and then subjected to analysis.

Samples taken for this analysis were obtained on Days 107, 129, 141, and 152 from the recarbonation effluent (sample location F). These samples, obtained after chemical treatment, were relatively free of suspended solids and yet had a relatively high mutagenic activity. Approximately 3.8 liters of each sample as obtained were passed through the XAD resins, and the acetone extracts so obtained were termed the "neutral fraction." This corresponds to the samples normally analyzed for mutagenic activity at the Richmond Laboratory. Another 3.8 liters of sample were acidified with 2.1 ml of concentrated

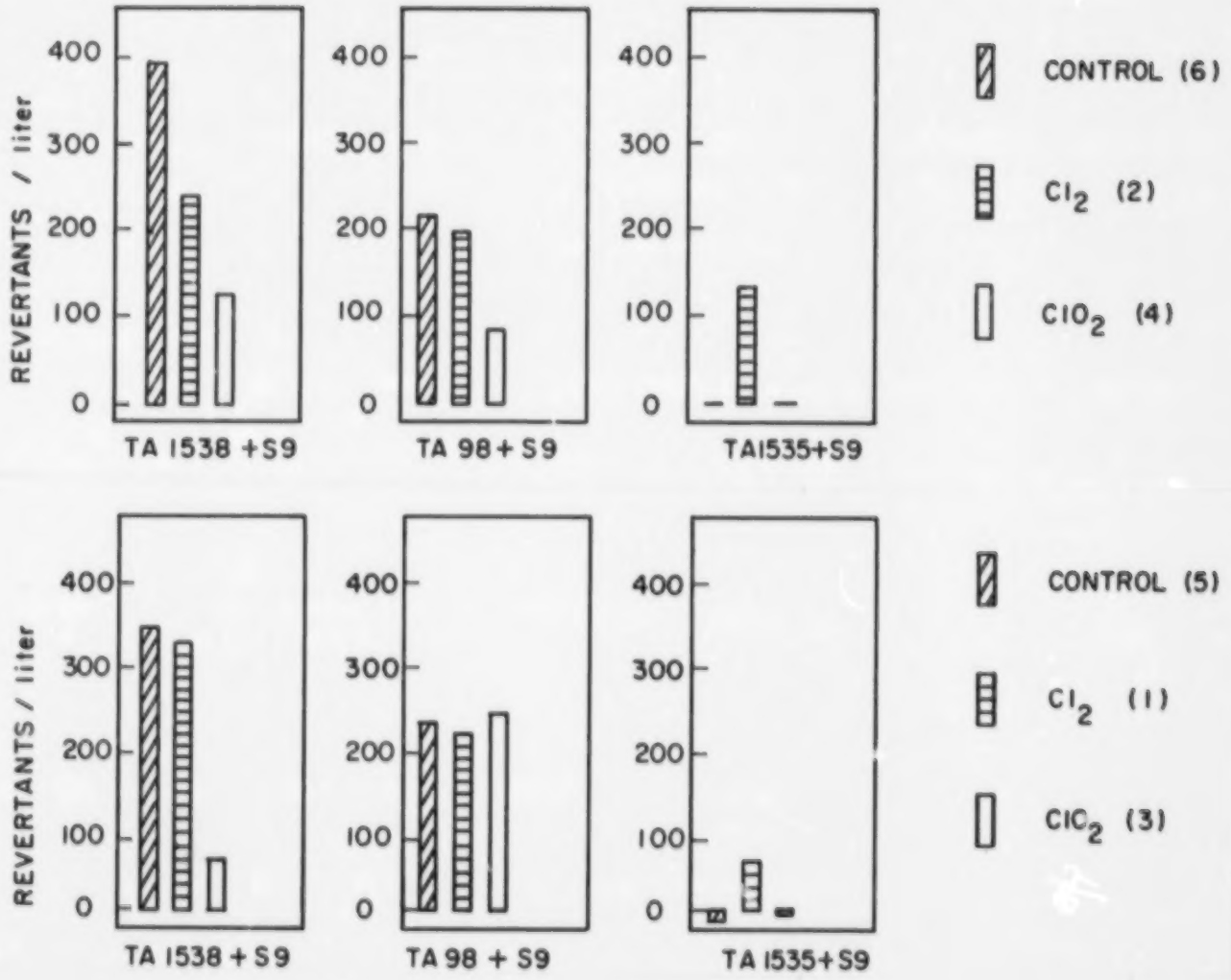


Figure 5. Comparison of mutagenic activity in secondary wastewater treated with chloride or chlorine dioxide.

sulfuric acid, passed through the XAD resins, and the acetone extracts obtained by this procedure were termed the "acid fraction." Samples of these extracts from Days 129 and 141 were sent to the Richmond Laboratory to compare the mutagenic activity with that of similar samples concentrated at the Richmond Laboratory. The results listed in Table 7 indicate that the activity of the neutral fractions (75 and 71 rev/mg) for strain TA98 with S9 were similar to that obtained with the Richmond extracts (Table 5, 60 and 60 rev/mg). The acid extracts also exhibited mutagenic activity, but somewhat smaller than for the neutral extracts.

TABLE 7. MUTAGENIC ACTIVITY OF RECARBONATION EFFLUENT (F) XAD-ACETONE EXTRACTS USED FOR DETAILED CHEMICAL CHARACTERIZATION

Day of Sampling	Type of Extract	Strain TA98				Strain TA1535			
		with S9		without S9		with S9		without S9	
		Rev/mg	Act.	Rev/mg	Act.	Rev/mg	Act.	Rev/mg	Act.
129	Neutral	75	2.9			NDR*			
	Acid	44	0.6	Toxic		NDR		Toxic	
141	Neutral	71	3.1			55	2.9	71	5.2
	Acid	15	0.7	Toxic		NDR		Toxic	

*NDR - not determined as greatest sample reversion rate was not more than twice the spontaneous reversion rate.

The compounds identified in these two different extracts are listed in Table 8. Several other compounds were present as indicated by peaks on the chromatograms, but the compounds could not be identified from their mass spectra alone. A detailed listing of the estimated molecular weights and the estimated wieghts of the base peaks for these compounds are given in Appendix D.

Many of the compounds listed in Table 8 are represented as the methyl esters, which result from the methylation procedure employed. The number of compounds determined by this procedure and listed in Table 8 or otherwise described in Appendix D is quite small and of limited usefulness for identifying those causing mutagenic activity in the samples. Most of the identified organic compounds are aromatic and aliphatic carboxylic acids, particularly the fatty acids. Most of the unidentified compounds appear to be chiefly aromatic in nature. In addition, many appear to be brominated (Appendix D).

The compounds identified represent only a small fraction of the total organic material present in the samples analyzed. It appears that the identification of the particular materials responsible for mutagenesis would require further separation of the extracted material into much smaller fractions that might be easier to resolve analytically. Even so, this could constitute a significant effort, much larger than that represented by this particular study.

TABLE 8. ORGANIC COMPOUNDS (AFTER METHYLATION) IDENTIFIED IN XAD-ACETONE EXTRACTS

Compound Identified	Molecular Weight	Present in Neutral Extract	Present in Acid Extract
A methylated furan	140		x
Methyl-3-methoxybenzoate	166		x
A naphthalene derivative	184		x
Dimethyl iso- or terephthalate	194		x
Ethyl or methyl terephthalate	208		
Methyl p-toluene sulfonate	186		x
Methyl clofibrate	228	x	x
Dimethyl iso- or terephthalate	194	x	x
Methyl laurate	214		x
Ethyl p-toluene sulfonate	200		x
Halogenated aromatic acid	218		x
Methylated or methoxylated aromatic	200		x
Aromatic carboxylic acid methyl ester	244		x
Methylated aromatic	214	x	x
p-toluenesulfonamide	199	x	x
A methyl tetradecanoate	242		x
Methyl myristate	242	x	x
Methyl pentadecanoate (3 isomers)	256	x	x
A methyl hexadecanoate	270		x
Methyl palmitoleate	268	x	x
Methyl palmitate	270	x	x
3,6-dimethylphenanthrene (Internal Std.)	206	x	x
Methyl heptadecanate	284		x
Methyl linoleate	284		x
Methyl stearate	298	x	x
Alkylated brominated aromatic	356		x
Methyl benzoate	136	x	
Methylated methoxylated aromatic	190	x	
Diethyl phthalate	222	x	
Pentachloroanisol	278	x	
Methyl linoleate	294	x	
Methyl oleanate	296	x	

MUTAGENIC ACTIVITY OF AQUIFER SAMPLES

Several 3.8-liter samples for mutagenic activity were taken of reclamation plant effluent just prior to injection into the 8-m deep aquifer, and from aquifer sampling wells located a short distance from the point of injection. Samples were taken as follows: Injection water (Days 19 and 56), sampling well P-5 (Days 56 and 73), sampling well P-6 (Day 73), and sampling well P-7 (Days 56 and 73). The XAD-acetone extracts obtained varied from 15 mg (P-5 on Day 56) to 45 mg (P-7 on Day 73), and were tested with five strains (TA98, TA100, TA1535, TA1537, and TA1538) both with and without S9. In no

case was the mutagenic activity greater than twice the spontaneous reversion rate for the strains.

Because of the low activity noted in the above samples, larger samples of injection water were collected and analyzed for mutagenic activity. The results are summarized in Table 9. The mass of XAD-acetone-extractable material obtained varied greatly, making it difficult to develop a routine testing protocol for the Ames assay. Because of the low activity of the fairly large amounts of extract tested, it was not possible to determine the dose-response relationship for any of the injection water extracts; therefore, the mutagenic activity reported is the apparent activity based on the concentration tested. Only on Day 114 was the number of revertants sufficiently high above the background level to enable terming the results positive. For all other samples the results were either negative or borderline. Because of the low mutagenic activity in the injection water, even with the larger samples, and the low activity as found previously with aquifer samples, attempts to concentrate and analyze larger samples of aquifer water were not made.

TABLE 9. MUTAGENIC ACTIVITY OF INJECTION WATER

Day of Sampling	Sample Vol., liters	Extract Obt. mg/l	Strain TA98 + S9				Strain TA1535 + S9			
			Rev/mg	Act.	Rev/l	Act/l	Rev/mg	Act.	Rev/l	Act/l
78	16	0.19	69	1.1	13	0.21	14	0.38	3	0.07
99	20	3.20	5	0.04	16	0.13	1.6	0.0	5	0.00
114	20	1.78	16	5.3	28	9.4	14	16	25	28
124	20	0.38	23	1.4	9	0.53	8	3.0	3	1.1
136	20	0.60	30	3.8	18	2.3	12	1.2	7	0.7

The results of these studies indicate that the mutagenic activity of Palo Alto secondary effluent was greatly decreased through advanced wastewater treatment. The activity was very low in the reclaimed water used for injection and in the groundwater aquifer itself near the point of injection, too low in general for positive detection in up to 20 liters of sample. It was originally planned to determine the ability of the aquifer to remove mutagenic materials from injected water, but because of the low activity found, this was not possible.

SUMMARY

Compounds capable of frameshift mutagenesis are present in the secondary effluent entering the Reclamation Plant at Palo Alto, California. With the exception of the granular activated-carbon unit, the advanced wastewater treatment processes appear not to effectively remove these compounds. Chlorination of the secondary effluent entering the reclamation plant almost always

led to the formation of new base-pair substitution mutagens. However, the unit processes following chlorination were able to remove these compounds from the wastewater.

The detailed characterization of XAD-acetone extracts indicated they contained many fatty acids, phthalates, and aromatic compounds. They also contained several unidentified compounds which contain bromine. The many potentially carcinogenic halogenated, but volatile compounds, present in the wastewater, however, were lost in the organic concentrations procedure and so were not susceptible to the mutagenic testing used.

NPOX concentration usually increase following chlorination of secondary effluent, and this increase was frequently accompanied by an increase in mutagenic activity in both the tester strains. However, increased mutagenic activity in chlorinated carbon effluents were not always accompanied by an increase in NPOX concentration. Although there may be some correlation between mutagenic activity and the concentration of halogen-containing organics in wastewater, there is insufficient data on which to draw a firm conclusion at this time.

Reclamation treatment, especially activated-carbon adsorption, was quite effective in reducing the mutagenic activity of Palo Alto secondary effluent. Injection water may contain compounds capable of causing mutagenesis, but because of the low concentration of organic material in the injection water, it was difficult to quantitate the presence of these compounds. Mutagenically active compounds were detected positively in only one 20-liter sample of injection water, and in none of the aquifer samples tested.

REFERENCES

1. Gehrs, C. W., et al. Effects of Chlorine-Containing Organics on Aquatic Environments. Nature, 249:675, 1974.

2. Jolley, R. L. Chlorine-Containing Organic Constituents in Chlorinated Effluents. J. Water Pollut. Control Fed., 47:601, 1975.

3. Saxena, J., and D. J. Schwartz. Mutagens in Wastewaters Renovated by Advanced Wastewater Treatment. Bull. Env. Contam. and Tox., 22:319, 1979.

4. McCarty, P. L., and M. Reinhard. Trace Organics Removal by Advanced Wastewater Treatment. J. Water Pollut. Control Fed., 52:1907, 1980.

5. McCarty, P. L., M. Reinhard, J. Graydon, J. Schreiner, K. Sutherland, T. Everhart, and D. G. Argo. Advanced Treatment for Wastewater Reclamation at Water Factory 21. Tech. Report No. 236, Dept. of Civil Engineering, Stanford University, January 1980.

6. Randtke, S., Jr., and P. L. McCarty. Removal of Soluble Secondary Effluent Organics. J. Env. Eng. Div., ASCE, (EE4):539, August 1977.

7. Chow, B. M., and P. V. Roberts. Halogenated By-Product Formation in Wastewater Treatment Using Chlorine and Chlorine Dioxide. Tech. Report No. 245, Dept. of Civil Engineering, Stanford University, February 1980.

8. Dobbs, R. A., R. H. Wise, and R. B. Dean. The Use of Ultra-Violet Absorbance for Monitoring the Total Organic Carbon Content of Water and Wastewater. Water Res., 6:1173, 1972.

9. Diehl, J. C., and S. W. Tromp. First Report on Geographical and Geological Distribution of Carcinogens in the Netherlands. The Leiden Foundation for the Study of Psychophysics, Leiden, The Netherlands, 1953.

10. Tromp, S. W. Schweizerische Zeitschrift für Allgemeine Pathologie, 18:929, 1955.

11. Stocks, P. Regional and Local Differences in Cancer Death Rates. Publ. No. 1, General Register Office, London, England, 1974.

12. Page, T., R. H. Harris, and S. S. Epstein. Drinking Water and Cancer Mortality in Louisiana. Science, 193:55, 1976.

13. Cantor, K. P., and L. J. McCabe. The Epidemiologic Approach to the Evaluation of Organics in Drinking Water. In: Water Chlorination, Environmental Impact and Health Effects, Vol. 2, R. J. Jolley, H. Gorchev, and D. H. Hamilton, Jr., eds. Ann Arbor Science Publishers, 1978. p. 379.

14. Alvanja, M., I. Goldstein, and M. Sussen. In: Water Chlorination, Environmental Impact and Health Effects, Vol. 2, R. J. Jolley, H. Gorchev, and D. H. Hamilton, Jr., eds. Ann Arbor Science Publishers, 1978. p. 395.

15. Pelon, W., B. F. Whitman, and T. W. Beasley. Env. Sci. Tech., 11:619, 1977.

16. Loper, J. C., D. R. Lang, R. S. Schoemy, B. R. Richmond, P. M. Gallagher, and C. C. Smith. J. Toxiology and Env. Health, 4:919, 1978.

17. Simmon, V. F., K. Kanhanen, and R. G. Tardiff. Mutagenic Activity of Chemicals Identified in Drinking Water. In: Progress in Genetic Toxicology, D. Scott, B. A. Bridges, and F. H. Sobels, eds. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands, 1977, p. 249.

18. Rappaport, S. M., R. Talcott, M. Hollstein, and M. Richard. Determination of Mutagenicity of Selected California Wastewaters. Final Report to Water Resources Control Board, State of California, Sacramento, California, Contract No. W6-131-40, June 2, 1978.

19. Ames, B. N., J. McCann, and E. Yamasaki. Mutation Research, 31:347, 1975.

20. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. Proc. of the National Academy of Sciences, 72:5135, 1975.

21. McCann, T., and B. N. Ames. Proc. of the National Academy of Sciences, 73:950, 1976.

22. APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 14th ed. American Public Health Assoc., Washington, D.C., 1976.

23. Henderson, J. E., G. R. Peyton, nd W. H. Glaze. A Convenient Liquid-Liquid Extraction Method for the Determination of Halomethanes in Water at the Parts-per-Billion Level. In: Identification and Analysis of Organic Pollutants in Water, L. H. Keith, ed. Ann Arbor Science Publ., Ann Arbor, Michigan, 1976.

24. Grob, K., and F. Zurcher. Stripping of Trace Organic Substances. J. Chromtog., 117:285, 1976.

APPENDIX A

COLLECTIVE PARAMETER RESULTS

TABLE A-1. COLLECTIVE PARAMETER RESULTS FOR UNCHLORINATED SECONDARY EFFLUENT (B)

Day of Analysis	COD mg/1	TOC mg/1	UV Absorb @ 254 µm per cm	TOX µg/1	POX µg/1	NPOX µg/1
54		> 7		266	173	93
65			0.136	149	61	88
76		11	0.143			
81						
86		11.5	0.143	177	72	105
92						
95		11	0.149			
99						
107	42.5					
	41.5		0.155	336	180	156
118	34.7					
	38.1	10.5	0.156	238	111	127
124						
129	39.9					
	39.9	11	0.152	193	76	117
135						
141	37.9					
	36.3	10.5	0.157	438	312	126
152	35.3					
	35.7	11	0.165	446	310	136
Geom. Mean	38.1	10.9	0.15	192	135	117
Spread Factor	1.07	1.03	1.06	2.10	1.91	1.21
95% CI	36.3-40.0	10.6-11.2	0.143-0.157	103-357	79-232	100-137

TABLE A-2. COLLECTIVE PARAMETER RESULTS FOR CHLORINATED SECONDARY EFFLUENT (C)

Day of Analysis	COD mg/l	TOC mg/l	UV Absorb @ 254 μm per cm	TOX μg/l	POX μg/l	NPOX μg/l
54		> 7		312	158	154
65			0.171	250	78	172
76		11	0.171	221	70	151
81						
86		7.5	0.157	282	79	203
92						
95		10.5	0.179			
99						
107	44.8 41.9	12.5	0.168	297	92	205
118	36.5 36.8	9	0.185	266	100	166
124						
129	50.6 52.2	13.5	0.207	283	150	133
135						
141	38.6 38.6	11.5	0.171	404	237	167
152	38.8 39.2	9	0.194	414	238	176
Geom. Mean	41.5	10.4	0.18	297	120	168
Spread Factor	1.14	1.21	1.09	1.23	1.61	1.15
95% CI	37.8- 45.6	8.9- 12.2	0.168- 0.192	253- 348	83- 173	151- 187

TABLE A-3. COLLECTIVE PARAMETER RESULTS FOR RECARBONATED EFFLUENT (F)

Day of Analysis	COD mg/l	TOC mg/l	UV Absorb @ 254 μm per cm	TOX μg/l	POX μg/l	NPOX μg/l
54						
65						
76						
81						
86						
92						
95						
99						
107	25.7 22.8	7.5	0.130	258	9	249
118	27.8 26.7	8	0.160	154	10	144
124	20.9 22.0	6	0.144			
129	22.8 21.4	7	0.155	134	3	131
135	27.2 30.0	9	0.167			
141	19.6 20.9	4	0.158	147	8	139
152	24.2 25.7	8.5	0.179	161	7	154
Geom. Mean	23.9	6.9	0.16	166	7	159
Spread Factor	1.14	1.32	1.11	1.29	1.62	1.30
95% CI	22.2- 25.8	5.3- 8.9	0.145- 0.176	121- 228	4- 13	115- 220

TABLE A-4. COLLECTIVE PARAMETER SUMMARY FOR OZONATED EFFLUENT (G)

Day of Analysis	COD mg/l	TOC mg/l	UV Absorb @ 254 μm per cm	TOX μg/l	POX μg/l	NPOX μg/l
54		> 7		172	63	109
65			0.139	181	38	143
76			0.134	205	93	112
81		8	0.133			
86		10	0.140	244	36	208
92		7	0.111			
95		8.5	0.114	231	23	208
99	20.6	7	0.076			
107						
118						
124						
129						
135						
141						
152						
Geom. Mean		8	0.12	205	45	150
Spread Factor		1.16	1.24	1.16	1.72	1.37
95% CI		6.7-9.6	0.098-0.146	171-246	23-88	101-222

TABLE A-5. COLLECTIVE PARAMETER SUMMARY FOR ACTIVATED-CARBON EFFLUENT (H)

Day of Analysis	COD mg/l	TOC mg/l	UV Absorb @ 254 μm per cm	TOX μg/l	POX μg/l	NPOX μg/l
54		2.5		20	10	10
65			0.016	27	15	12
76			0.024	47	16	31
81		2.8				
86		4.2	0.030	92	18	74
92		1.8	0.016			
95		1.9	0.018	123	13	110
99	10.4	1.5	0.031			
107	8.6 7.4	1.4	0.016	243	15	228
118						
124	10.0 10.6	3.8	0.049			
129	9.0 9.2	3.6	0.042	97	11	86
135	13.7 13.9	5.0	0.050			
141	12.0 10.4	1.1	0.046	108	7	101
152	14.9 11.8	4.6	0.051	66	7	59
Geom. Mean	10.7	2.5	0.03	71	12	53
Spread Factor	1.23	1.67	1.62	2.18	1.42	2.84
95% CI	9.5- 12.1	1.8- 3.5	0.022- 0.041	39- 129	9- 16	24- 118

TABLE A-6. COLLECTIVE PARAMETER SUMMARY FOR CHLORINATED RECLAMATION PLANT EFFLUENT (K)

Day of Analysis	COD mg/l	TOC mg/l	UV Absorb @ 254 µm per cm	TOX µg/l	POX µg/l	NPOX µg/l
54		2.3		19	9	10
65			0.042	30	21	9
76		1.7	0.076	46	19	27
81						
86		2.8	0.034	141	7	134
92						
95		1.9	0.046			
99						
107	8.4 6.3	2.1	0.030	226	10	216
118	5.6 5.0	1.6	0.068	61	7	54
124						
129	8.7 8.7	3.0	0.069	60	8	52
135						
141	8.8 8.5	2.0	0.061	77	12	65
152	9.6 10.9	3.2	0.076	84	8	76
Geom. Mean	7.8	2.2	0.05	65	10	46
Spread Factor	1.28	1.28	1.43	2.11	1.51	2.93
95% CI	6.5- 9.3	1.8- 2.7	0.038- 0.066	37- 115	7- 14	20- 105

APPENDIX B

VOA RESULTS

TABLE B-1. VOA RESULTS FOR UNCHLORINATED SECONDARY EFFLUENT (B)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54	12.5	0.23	0.09	< 0.05	< 0.05	59	35	103
65	8.07	0.21	0.13	< 0.05	0.20	38	11	27
71								
76	11.6	0.27	0.08	< 0.05	0.17	19	10	28
81								
86	11.6	0.33	0.12	< 0.05	0.15	36	14	15
92								
95	18.4	0.33	0.15	< 0.05	< 0.05	45	32	24
99								
107	14.9	0.83	0.33	< 0.05	0.55	57	59	39
118	13.8	0.45	0.20	0.14	< 0.05	126	15	29
124								
129	11.8	< 0.05	0.04	< 0.05	< 0.05	37	18	42
135								
141	15.4	< 0.05	0.16	< 0.05	< 0.05	246	66	190
152	15.0	< 0.05	0.23	< 0.05	< 0.05	286	47	110
Geom. Mean	13.0	0.23	0.13	< 0.05	0.10	65	25	44
Spread Factor	1.26	2.19	1.87	?	2.90	2.45	2.07	2.67
95% CI	11.1-15.3	0.11-0.51	0.09-0.21	?	0.02-0.42	34-122	15-41	25-79

TABLE B-2. VOA RESULTS FOR UNCHLORINATED SECONDARY EFFLUENT (C)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54	13.8	0.90	0.25	< 0.05	< 0.05	48	32	76
65	11.9	2.04	0.87	< 0.05	0.17	25	13	40
71								
76	15.6	1.81	0.53	< 0.05	0.32	22	12	35
81								
86	13.8	1.77	0.51	< 0.05	0.18	37	14	14
92								
95	22.5	2.89	0.85	< 0.05	< 0.05	48	35	27
99								
107	15.5	1.60	0.49	0.19	0.37	43	53	31
118	16.5	3.04	1.13	0.52	0.89	155	17	32
124								
129	20.5	1.65	0.35	0.96	4.36	61	28	77
135								
141	15.1	0.58	0.28	< 0.05	< 0.05	224	68	163
152	19.3	3.64	0.35	0.20	3.64	292	53	112
Geom. Mean	16.2	1.63	0.50	0.10	0.2	65	27	47
Spread Factor	1.23	1.70	1.70	4.50	8.95	2.49	1.93	2.17
95% CI	14.0-18.7	1.1-2.4	0.34-0.72	0.01-0.8	0.04-1.3	34-123	17-43	27-82

TABLE B-3. VOA RESULTS FOR RECARBONATED EFFLUENT (F)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54								
65								
71								
76								
81								
86								
92								
95								
99								
107	0.65	0.09	0.28	< 0.05	< 0.05	0.51	0.95	0.16
118	0.87	0.17	0.11	0.12	< 0.05	3.33	0.31	0.46
124	0.74	0.13	0.08	0.15	< 0.05	0.39	0.41	0.31
129	4.21	0.26	0.07	0.04	< 0.05	0.57	0.09	0.12
135	0.67	0.06	< 0.05	< 0.05	< 0.05	0.19	0.36	0.85
141	1.21	0.08	< 0.05	< 0.05	0.07	1.04	0.23	0.82
152	2.30	0.17	0.21	< 0.05	0.17	1.12	0.60	0.80
Geom. Mean	1.2	0.12	0.09	0.03	< 0.05	0.7	0.34	0.4
Spread Factor	2.04	1.73	2.43	3.42	?	2.64	2.20	2.29
95% CI	0.63-2.2	0.08-0.20	0.03-0.25	0.003-0.3	?	0.3-1.7	0.17-0.69	0.19-0.83

TABLE B-4. VOA SUMMARY FOR OZONATED EFFLUENT (G)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54	10.6	1.04	0.31	< 0.05	< 0.05	17	19	24
65	11.0	1.87	0.71	< 0.05	< 0.05	13	8.1	18
71	14.8	2.54	0.55	< 0.05	< 0.05	17	8.5	23
76	13.3	1.40	0.49	< 0.05	< 0.05	7.8	6.2	17
81	13.2	1.77	0.45	< 0.05	0.10	7.3	7.6	12
86	8.1	1.13	0.34	< 0.05	< 0.05	8.1	4.1	3.8
92	0.58	< 0.05	< 0.05	< 0.05	< 0.05	0.16	< 0.05	0.13
95	1.44	< 0.05	< 0.05	< 0.05	< 0.05	0.79	0.67	0.47
99	0.66	< 0.05	< 0.05	< 0.05	< 0.05	0.20	< 0.05	0.24
107								
118								
124								
129								
135								
141								
152								
Geom. Mean	4.80	1.2	0.36	< 0.05	< 0.05	3.3	3.6	3.6
Spread Factor	3.61	1.67	1.60	?	?	6.17	3.48	7.97
95% CI	1.8-12.6	0.72-2.0	0.23-0.58	?	?	0.8-13	1.2-11	0.76-17

TABLE B-5. VOA SUMMARY FOR ACTIVATED-CARBON EFFLUENT (H)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54	0.47	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
65	0.23	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.11
71	0.86	2.14	< 0.05	< 0.05	< 0.05	6.5	0.54	21.2*
76	1.34	< 0.05	< 0.05	< 0.05	< 0.05	0.25	0.17	0.27
81	1.47	< 0.05	< 0.05	< 0.05	< 0.05	0.21	< 0.05	0.28
86	1.81	< 0.05	< 0.05	< 0.05	< 0.05	0.24	< 0.05	0.15
92	2.11	< 0.05	< 0.05	< 0.05	< 0.05	0.23	< 0.05	0.11
95	2.24	< 0.05	< 0.05	< 0.05	< 0.05	0.28	< 0.05	0.15
99	3.11	< 0.05	< 0.05	< 0.05	< 0.05	0.39	< 0.05	0.18
107	2.71	0.13	0.59	< 0.05	< 0.05	0.44	0.39	0.40
118								
124	3.98	0.14	< 0.05	< 0.05	< 0.05	0.49	< 0.05	0.04
129	4.35	0.22	< 0.05	< 0.05	< 0.05	0.60	< 0.05	0.06
135	5.67	0.26	0.06	< 0.05	0.05			
141	6.73	0.29	< 0.05	< 0.05	0.12	1.10	< 0.05	0.31
152	6.42	0.08	< 0.05	< 0.05	0.08	1.16	< 0.05	0.42
Geom. Mean	2.1	0.04	< 0.05	< 0.05	0.02	0.4	0.06	0.17
Spread Factor	2.66	7.89	?	?	2.91	3.26	3.84	2.13
95% CI	1.2-3.6	0.005-0.31	?	?	0.003-0.14	0.2-0.8	0.005-0.7	0.10-0.27

*Data excluded from summary calculations.

TABLE B-6. VOA SUMMARY FOR CHLORINATED RECLAMATION PLANT EFFLUENT (K)
(Concentrations in µg/l)

Day of Anal.	Chloro-form	Bromodi-chloro-methane	Dibromo-chloro-methane	Tri-bromo-methane	Carbon tetra-chloride	1,1,1-trichloro-ethane	Tri-chloro-ethylene	Tetra-chloro-ethylene
54	0.34	< 0.05	< 0.05	< 0.05	0.88	< 0.05	< 0.05	0.10
65	0.29	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.13
71								
76	1.31	< 0.05	< 0.05	< 0.05	< 0.05	0.26	0.16	0.30
81								
86	2.13	< 0.05	< 0.05	< 0.05	< 0.05	0.18	< 0.05	0.14
92								
95	2.14	< 0.05	< 0.05	< 0.05	0.85	0.25	< 0.05	0.16
99								
107	3.55	0.13	0.10	0.61	< 0.05	0.43	0.04	0.08
118	5.40	0.25	0.06	< 0.05	0.06	0.74	0.02	2.69*
124								
129	6.31	0.44	0.13	< 0.05	< 0.05	0.93	< 0.05	0.11
135								
141	7.69	0.72	0.28	< 0.05	0.34	1.09	0.10	0.67
152	8.32	0.85	< 0.05	< 0.05	0.35	1.34	< 0.05	0.79
Geom. Mean	2.3	0.15	0.04	< 0.05	0.10	0.4	0.01	0.16
Spread Factor	3.41	3.68	3.53	?	5.31	2.73	5.92	1.99
95% CI	1.0-5.5	0.03-0.66	0.007-0.23	?	0.02-0.7	0.2-0.8	0.001-0.1	0.09-0.29

*Data excluded from summary calculations.

APPENDIX C.

CLSA RESULTS

TABLE C-1. CLSA RESULTS FOR UNCHLORINATED SECONDARY EFFLUENT (B)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
54											
65				0.64	4.8	3.1	4.7	10.1	3.9		
71											
76	< 0.01	< 0.01	< 0.01	0.12	2.6	2.4	1.9	13.1	5.3	< 0.01	< 0.01
81											
86				0.39	6.9	8.7	11.4	10.1	3.5		
92											
95	0.06	0.34	0.08	0.34	2.0	2.5	2.0	12.5	2.3	< 0.01	< 0.01
99											
107				0.24	4.1	5.2	9.6	17.1	4.5	< 0.01	< 0.01
118	0.28	0.76	0.16	1.07	7.5	14.9	16.6	17.5	4.3	0.17	0.22
124											
129	0.02	0.08	0.04	0.04	0.2	0.4	0.7	2.7	0.8		
135											
141			< 0.01	0.24	4.1	5.4	13.3	5.2	1.8	< 0.01	< 0.01
152	0.20	0.34	< 0.01	0.59	1.7	3.9	12.5	42.3	9.4	< 0.01	< 0.01
Geom. Mean	0.06	0.20	0.03	0.29	2.7	3.6	5.4	11.3	3.3	< 0.01	< 0.01
Spread Factor	5.0	3.35	3.56	2.76	2.86	2.8	2.06	2.23	2.08	?	?
95% CI	0.006-0.5	0.04-1.10	0.003-0.33	0.14-0.62	1.2-5.9	1.7-7.8	2.3-12.5	6.2-20.6	1.9-5.7	?	?
Range	< 0.01-0.28	< 0.01-0.76	< 0.01-0.16	0.04-1.07	0.20-7.5	0.40-14.9	0.7-16.6	2.7-42.3	0.8-9.4	< 0.01-0.17	< 0.01-0.22

TABLE C-1 cont.

TABLE C-1 cont.

Day of Anal.	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanide	Nonyl-cyanide
54						
68		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
71						
76	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
81						
86		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
92						
95	< 0.01	< 0.01	< 0.01	< 0.01	0.11	< 0.01
99						
107	< 0.01	< 0.01	0.07	0.04	0.14	< 0.01
118	< 0.01	0.28	0.02	0.06	0.15	< 0.01
124						
129	0.10	0.18	0.30	0.10	0.49	0.05
135						
141	< 0.01	0.06	< 0.01	< 0.01	0.14	< 0.01
152	0.86	0.20	< 0.01	0.10	0.09	< 0.01
Geom. Mean	< 0.01	0.06	0.004	0.04	0.10	< 0.01
Spread Factor	?	2.99	18.1	2.12	2.31	?
95% CI	?	0.01-0.3	$2(10)^{-5}$-0.85	0.01-0.11	0.04-0.24	?
Range	< 0.01-0.86	< 0.01-0.28	< 0.01-0.3	< 0.01-0.1	< 0.01-0.49	< 0.01-0.05

TABLE C-2. CLSA RESULTS FOR CHLORINATED SECONDARY EFFLUENT (C)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
54					4.5	2.8	3.7	7.5	2.6		
65	1.3	3.8		4.1	6.2	3.8	4.1	20.0	6.7		
71											
76	0.64	1.3		1.6	2.7	3.3	3.3	11.9	4.5	0.32	0.18
81											
86				0.30	7.2	9.8	13.3	10.4	3.2	< 0.01	< 0.01
92											
95	0.07		0.05	0.81	2.0	3.0	2.6	12.1		< 0.01	< 0.01
99											
107	< 0.01	0.26	0.09	0.14	2.5	3.2	6.0	11.4	1.2		
118	0.30	1.67	0.06	1.43	6.7	13.7	17.1	16.1	3.9	< 0.01	< 0.01
124											
129											
135											
141	0.25	0.76	0.26	0.43	5.4	7.8	18.0	7.2	2.4		
152	0.70	1.16	< 0.01	1.70	1.6	3.4	8.3	35.9	7.3	< 0.01	< 0.01
Geom. Mean	0.29	1.11	0.07	0.83	3.8	4.7	6.7	13.0	3.5	< 0.01	< 0.01
Spread Factor	3.57	2.58	2.70	3.19	1.79	1.77	2.15	1.67	1.86	?	?
95% CI	0.08-1.0	0.43-2.87	0.017-0.27	0.32-2.2	2.4-5.9	3.1-7.3	3.8-11.9	8.8-19.1	2.1-5.7	?	?
Range	< 0.01-1.3	0.26-3.8	< 0.01-0.26	0.14-4.1	1.6-7.2	2.8-13.7	2.6-18	7.2-35.9	1.2-7.3	< 0.01-0.32	< 0.01-0.18

TABLE C-2 cont.

TABLE C-2 cont.

Day of Analysis	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanide	Nonyl-cyanide
54		< 0.01	< 0.01	0.24	0.47	0.12
65	< 0.01		0.26	0.38	2.3	0.52
71						
76	2.4	1.02	2.3	0.60	2.3	0.32
81						
86	0.80	0.27	0.50	0.24	0.68	0.24
92						
95	< 0.01	0.19	0.37	0.13	0.44	0.18
99						
107	1.04	0.32	0.74	0.18	1.23	0.20
118		0.23	< 0.01	0.16	0.82	0.28
124						
129						
135						
141	0.35	1.33	1.84	0.58	1.86	0.46
152		0.28	0.50	0.32	1.02	0.22
Geom. Mean	0.51	0.33	0.47	0.28	1.04	0.26
Spread Factor	3.41	2.47	3.03	1.77	1.94	1.63
95% CI	0.09-2.8	0.15-0.73	0.18-1.26	0.18-0.42	0.63-1.71	0.18-0.37
Range	< 0.01-2.4	< 0.01-1.33	< 0.01-2.3	0.13-0.6	0.44-2.3	0.12-0.52

TABLE C-3. CLSA RESULTS FOR RECARBONATED EFFLUENT (F)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
57											
65											
71											
76											
81											
86											
92											
95											
99											
107	< 0.01	0.04	0.04	0.22	0.20	0.28	0.56	1.40	0.74	0.12	0.02
118	0.09	0.40	0.04	0.70	0.82	1.71	4.18	2.14	0.92	< 0.01	< 0.01
124	0.02	0.05	0.07	0.38	0.14	0.42	0.50	1.32	1.66		
129	0.05	0.10	0.04	0.16	0.09	0.18	0.26	1.35	0.48	0.07	
135	0.09	0.29	0.11	0.31	0.25	0.26	0.62	1.92	2.80	0.44	0.04
141	< 0.01	< 0.01		0.13	0.07	0.18	0.32	0.32	0.24	0.06	0.06
152	0.06	0.11		0.18	0.12	0.30	0.55	1.59	2.10	0.41	0.03
Geom. Mean	0.04	0.09	0.05	0.25	0.17	0.34	0.61	1.27	0.96	0.11	0.03
Spread Factor	2.25	3.23	1.57	1.85	2.32	2.09	2.33	1.77	2.53	3.34	1.91
95% CI	0.015-0.10	0.028-0.29	0.033-0.092	0.15-0.44	0.08-0.36	0.18-0.65	0.29-1.31	0.76-2.11	0.42-2.20	0.03-0.46	0.01-0.07
Range	< 0.01-0.09	< 0.01-0.4	0.04-0.11	0.13-0.7	0.07-0.82	0.18-1.71	0.26-4.18	0.32-2.14	0.24-2.8	< 0.01-0.44	< 0.01-0.06

TABLE C-3 cont.

TABLE C-3 cont.

Day of Analysis	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanide	Nonyl-cyanide
54						
65						
71						
76						
81						
86						
92						
95						
99						
107	0.10	0.10	0.11	0.06	0.15	0.10
118	0.06	0.08	0.22	0.06	0.22	0.09
124	0.04	0.04	0.08	0.06	0.14	0.07
129	0.23	0.06	0.07	0.04	0.14	0.04
135	0.12	0.04	0.06	0.08	0.10	< 0.01
141	0.08	0.04	0.04		0.02	0.03
152	< 0.01	0.13	0.11	0.08	0.14	0.06
Geom. Mean	0.08	0.06	0.09	0.06	0.11	0.05
Spread Factor	2.18	1.64	1.78	1.29	2.01	1.80
95% CI	0.03-0.16	0.04-0.10	0.05-0.14	0.05-0.08	0.06-0.20	0.03-0.09
Range	< 0.01-0.23	0.04-0.13	0.04-0.22	0.04-0.08	0.02-0.22	< 0.01-0.1

TABLE C-4. CLSA RESULTS FOR OZONATED EFFLUENT (G)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
54				3.2	2.2	1.2	15.1	7.8	3.1	1.3	< 0.01
65		8.7		1.8	5.6	3.4	12.0	3.4	1.5		
71	0.08	0.19	0.04	0.57	2.1	3.1	6.7	3.0	1.2	0.5	< 0.01
76	2.0	5.0		5.9	1.8	2.4	5.5	6.5	2.8	0.90	0.28
81	0.47	0.94	< 0.01	1.6	4.6	4.6	8.1	6.9	2.9	0.84	< 0.01
86	< 0.01	< 0.01	< 0.01	0.49	4.0	7.0	21.6	2.5	0.32	0.30	0.23
92	0.03		0.03	0.30	0.08	0.33	0.28	0.3	0.34	0.09	0.29
95	0.08	0.24	0.26	0.46	0.16	0.39	0.58	1.1	2.9		
99	< 0.01	< 0.01	< 0.01	0.24	0.06	0.10	0.30	0.22	0.22	0.06	< 0.01
107											
118											
124											
129											
135											
141											
152											
Geom. Mean	0.06	0.39	0.02	0.93	0.95	1.32	3.4	2.1	1.1	0.36	0.22
Spread Factor	11.7	12.3	7.9	3.18	5.91	4.35	5.53	3.80	2.90	3.43	1.24
95% CI	0.004-1.1	0.02-7.0	0.004-0.7	0.39-2.2	0.25-3.6	0.44-4.0	0.93-12.2	0.75-5.6	0.51-2.53	0.12-1.08	0.15-0.33
Range	< 0.01-2.0	< 0.01-8.7	< 0.01-0.26	0.24-5.9	0.06-5.6	0.1-7.0	0.28-21.6	0.22-7.8	0.06-3.1	0.06-1.3	< 0.01-0.29

TABLE C-4 cont.

TABLE C-4 cont.

Day of Analysis	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanid.	Nonyl-cyanide
54	45.6	1.2	6.6	2.8	15.8	2.1
65			1.2	0.68	3.3	0.52
71	12.8	0.80	2.2	0.60	2.1	0.36
76	12.5	< 0.01	0.44	< 0.01	0.48	0.18
81	7.5	1.2	2.4	0.86	3.2	1.05
86	8.5	6.8	3.3	0.58	2.2	0.49
92	1.8	0.89	1.6	0.20	1.1	0.66
95	1.0	0.37	0.44	0.17	0.42	0.16
99	0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
107						
118						
124						
129						
135						
141						
152						
Geom. Mean	3.8	0.71	1.29	0.37	1.5	0.5
Spread Factor	8.39	3.66	3.14	3.53	4.12	2.48
95% CI	0.67-21	0.20-2.6	0.51-3.3	0.12-1.1	0.46-4.6	0.24-1.05
Range	0.05-45.6	< 0.01-6.8	< 0.01-6.6	< 0.01-2.8	< 0.01-15.8	0.16-2.1

TABLE C-5. CLSA RESULTS FOR ACTIVATED-CARBON EFFLUENT (H)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
54				0.16	0.04	0.12	0.18	0.37	0.07	0.53	< 0.01
65			< 0.01	0.18	< 0.01	0.04	< 0.01	0.06		< 0.01	< 0.01
71	< 0.01	< 0.01	< 0.01	0.09	< 0.01	0.03	< 0.01	0.02	< 0.01	< 0.01	< 0.01
76	< 0.01	< 0.01		0.10	0.02	0.14	0.20	0.20	0.02	< 0.01	0.12
81	< 0.01	< 0.01	< 0.01	0.16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.04	< 0.01
86	< 0.01	< 0.01	< 0.01	0.17	< 0.01	< 0.01	< 0.01	< 0.01	0.06	< 0.01	< 0.01
92	< 0.01	< 0.01	< 0.01	0.07	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01
95				0.14	0.05	0.11	0.15	0.89	0.10	0.03	< 0.01
99	< 0.01	< 0.01	< 0.01	0.17	< 0.01	0.03	< 0.01	0.02	0.02	0.23	< 0.01
107	< 0.01	0.02	< 0.01	0.10	< 0.01	< 0.01	0.05	0.02	0.04	0.02	< 0.01
118											
124	0.02		0.02	0.28	< 0.01	0.10	0.06	0.04	0.04		
129	< 0.01	< 0.01	< 0.01	0.12	< 0.01	< 0.01	< 0.01	0.02	0.06	0.04	< 0.01
135	< 0.01	0.04	0.02	0.20	0.04	< 0.01	0.06	0.12	0.20	0.06	0.02
171	< 0.01	< 0.01	< 0.01	0.12	2.0	2.62	6.35	2.54	0.89	< 0.01	< 0.01
152	< 0.01	< 0.01	< 0.01	0.12	< 0.01	0.03	< 0.01	0.04	0.20	< 0.01	< 0.01
Geom. Mean*	< 0.01	< 0.01	< 0.01	0.14	0.02	0.03	0.04	0.04	0.04	0.02	< 0.01
Spread Factor*	?	?	?	1.46	2.07	2.88	2.98	5.89	3.01	6.24	?
95% CI*	?	?	?	0.11-0.17	0.006-0.04	0.01-0.07	0.01-0.12	0.01-0.11	0.02-0.09	0.004-0.09	?
Range*	< 0.01-0.02	< 0.01-0.04	< 0.01-0.02	0.07-0.28	< 0.01-0.05	< 0.01-0.14	< 0.01-0.20	< 0.01-0.89	< 0.01-0.2	< 0.01-0.53	< 0.01-0.12

TABLE C-5 cont.

TABLE C-5 cont.

Day of Analysis	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanide	Nonyl-cyanide
54	0.12	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
65	0.16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
71	0.07	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
76	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
81	0.26	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
86	0.18	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
92	0.30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
95	0.05	< 0.01	< 0.01	0.18	< 0.01	< 0.01
99	0.06	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
107	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
118						
124	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
129	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
135	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
141	0.02					
152	0.06	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Geom Mean*	0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Spread Factor*	3.43	?	?	?	?	?
95% CI*	0.02-0.12	?	?	?	?	?
Range*	< 0.01-0.3	< 0.01	< 0.01	< 0.01-0.18	< 0.01	< 0.01

*Excludes data from 9th week.

TABLE C-6. CLSA RESULTS FOR CHLORINATED RECLAMATION PLANT EFFLUENT (K)
(Concentrations in µg/l)

Day of Anal.	p-xylene	m-xylene	Ethyl-benzene	Chloro-benzene	1,3-di-chloro-benzene	1,4-di-chloro-benzene	1,2-di-chloro-benzene	1,2,4-tri-chloro-benzene	Naph-thalene	Unknown X	Unknown Y
54	< 0.01	< 0.01	< 0.01	0.12	< 0.01	< 0.01	< 0.01	< 0.01	0.11	0.04	< 0.01
65	0.03		< 0.01	0.13	< 0.01	0.03	0.08		0.08	0.02	< 0.01
71											
76	< 0.01	< 0.01	< 0.01	0.30	< 0.01	< 0.01	< 0.01	< 0.01	0.12	0.06	< 0.01
81											
86											
92											
95	< 0.01	< 0.01	< 0.01	0.20	< 0.01	0.07	0.06	0.06		< 0.01	< 0.01
99											
107	< 0.01	0.02	< 0.01	0.13	0.22	0.25	0.49	1.02	0.38	< 0.01	< 0.01
118		0.05	< 0.01	0.30	0.61	1.08	1.60	1.50	0.51	< 0.01	< 0.01
124											
129	0.02	0.05	0.04	0.33	0.04	0.16	0.29	0.90	0.60		
135											
141											
152				0.16	0.05	0.08	0.14	0.58	0.52	< 0.01	< 0.01
Geom. Mean	< 0.01	0.02	< 0.01	0.19	0.02	0.07	0.12	0.28	0.25	0.02	< 0.01
Spread Factor	?	2.20	?	1.53	9.79	5.86	5.85	4.84	2.38	2.86	?
95% CI	?	0.005-0.09	?	0.14-0.27	0.001-0.58	0.01-0.42	0.02-0.69	0.05-1.71	0.12-0.55	0.003-0.11	?
Range	< 0.01-0.03	< 0.01-0.05	< 0.01-0.04	0.12-0.33	< 0.01-0.61	< 0.01-1.08	< 0.01-1.6	< 0.01-1.5	0.08-0.6	< 0.01-0.06	< 0.01-

TABLE C-6 cont.

TABLE C-6 cont.

Week of Analysis	Heptal-aldehyde	Pentyl-cyanide	Hexyl-cyanide	Heptyl-cyanide	Octyl-cyanide	Nonyl-cyanide
54	0.90	< 0.01	0.13	< 0.01	< 0.01	< 0.01
65	0.30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
71						
76	0.53	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
81						
86						
92						
95	0.06	< 0.01	< 0.01	0.02	< 0.01	< 0.01
99						
107	0.03	< 0.01	0.02	0.02	0.04	0.04
118	0.03	< 0.01	< 0.01	< 0.01	0.04	0.04
124						
129	0.16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
135						
141						
152	0.04	0.03	0.03	0.02	0.05	0.04
Geom. Mean	0.12	< 0.01	0.007	< 0.01	< 0.01	< 0.01
Spread Factor	4.02	?	7.32	?	?	?
95% CI	0.04-0.4	?	0.0002-0.27	?	?	?
Range	0.03-0.9	< 0.01-0.03	< 0.01-0.13	< 0.01-0.02	< 0.01-0.05	< 0.01-0.04

APPENDIX D

MATERIALS DETECTED AND IDENTIFIED BY GC/MS

TABLE D-1. Acid Acetone Extracts of Recarbonation Effluent

Stanford Ref.#	Compound Name	Formula	MW	Base P.	Evid.
321			140?	45	
322	A methylated furan ?		140	109	
219		C_8H_8O	136	105	1
351			146	45	
361				45	
365				57	
367				83	
300	Methyl 3-methoxy-benzoate	$C_9H_{10}O_3$	166	135	2B
330	Naphthalene derivative		184	169	
340	Methoxylated or hydroxymethylated		198	167	
335	Aromatic		194	179	
333	Aromatic		192	77	
302	Dimethyl iso- or terephthalate	$C_{10}H_{10}O_4$	194	163	2B
336	Methylated and methoxylated or hydroxymethylated		196	150	
301	Methyl p-toluenesulfonate	$C_8H_{10}SO_3$	186	91	2A
307	Methyl clofibrate	$C_{11}H_{13}ClO_3$	228	128	1
307	Dimethyl iso- or terephthalate	$C_{10}H_{10}O_4$	194	163	2B
234	Methyl laurate	$C_{13}H_{26}O_2$	214	74	
318	Ethyl p-toluenesulfonate	$C_9H_{12}O_3$	200	91	2A
318	Halogenated aromatic acid		218		
320b	Methylated or methoxylated aromatic		200	105	
320b	Aromatic carboxylic acid methyl ester		244		
320b					
331	Aromatic		190	119	
319	Aromatic, methylated		214	199	
303	p-toluenesulfonamide	$C_9H_{13}NSO_2$	199	91	2C

TABLE D-1 cont.

TABLE D-1 cont.

Stanford Ref.#	Compound Name	Formula	MW	Base P.	Evid.
308	A methyl tetradecanoate	$C_{15}H_{30}O_2$	242	74	1
344			240	75	
240	Methyl myristate	$C_{15}H_{30}O_2$	242	74	1
309	A methyl pentadecanoate	$C_{16}H_{32}O_2$	256	74	1
310	A methyl dentadecanoate	$C_{16}H_{32}O_2$	256	74	1
375	Aromatic	mono-brominated		227	
332	Alkylated aromatic		190	105	
311	A methyl pentadecanoate, probably methyl m-pentadecanoate	$C_{16}H_{32}O_2$	256	74	1
34	Appears to be aromatic		232	232	
313	A methyl hexadecanoate	$C_{17}H_{34}O_2$	270	74	1
312	Methyl palmitoleate	$C_{17}H_{32}O_2$	268	55	1
347	Appears to be a methyl ester of an alkylated aromatic acid		278	207	
244	Methyl palmitate	$C_{17}H_{34}O_2$	270	74	1
304*	3,6-dimethylphenanthrene	$C_{16}H_{14}$	206	206	1
314	A methyl heptadecanoate	$C_{18}H_{36}O_2$	284	74	1
315	Methyl linoleate	$C_{19}H_{34}O_2$	284	41	1
376	Branched chain or unsaturated hydrocarbon			43	
348	Homologous to spectrum #569. Substance 569 + CH_{20} ?		308	207	
246	Methyl stearate	$C_{19}H_{38}O_2$	298	74	1
350	Alkylated, brominated aromatic		356	287	
377				43	
349	Probable precursor of spectrum #775, 779, 785, 823, 326, 353, 866, or 940	probably $C_{19}H_{30}O_4$ by HRMS	322	117	
378	Homolog of aromatic spectrum #660			43	
379				117	
380	Perhaps brominated			117	
381				43	
382				117	
384	Monobrominated			117	
351	Monobrominated		400	117	
352	Monobrominated, isomer of		400	117	
385	Monobrominated			131	
386	Monobrominated			117	
387	Monobrominated			117	

*Internal Standard

TABLE D-2. Neutral Acetone Extracts of Recarbonation Effluent

Stanford Ref.#	Compound Name	Formula	MW	Base P.	Evid.
353				43	
354				43	
219	Methylbenzoate	$C_8H_8O_2$	136	105	1
324			154	43	
325	Isomer of spectrum #64		154	43	
355			98?	83	
323			140	43	
356				43	
358				59	
359				43	
360				43	
362				43	
328	Homolog of spectrum #97		168	43	
363				43	
364				59	
326			154	57	
327			154	43	
369				43	
337			197?	43	
338	Isomer of spectrum #337		197?	43	
368			126?	55	
369			112	43	
334				83	
370				43	
339			198	43	
371				43	
372				43	
342	Appears to be homolog of spectrum #64 and 67		212?	43	
302	Dimethyl iso- or terephthalate	$C_{10}H_{10}O_4$	194	163	2B
329	Perhaps homolog of spectrum #255			55	
307	Methyl clofibrate	$C_{11}H_{13}ClO_3$	228	128	2B
307	Dimethyl iso- or terephthalate	$C_{10}H_{10}O_4$	194	163	2C
339			198	43	
305	Terephthalic acid, ethyl methyl ester	$C_{11}H_{12}O_4$	208	163	2C
331	Aromatic methylated or methoxylated		190	119	
320b	Aromatic		200	105	
306		$C_{12}H_{14}O_4$	222	149	1
373	Appears to be homologous to spectrum #336			43	

TABLE D-2 cont.

TABLE D-2 cont.

Stanford Ref.#	Compound Name	Formula	MW	Base P.	Evid.
303	p-toluenesulfonamide	$C_9H_{13}NSO_2$	199	91	2C
319	Methylated aromatic		214	199	
345	Methoxylated aromatic		244	167	
374				43	
46	Pentachloroanisole	$C_7H_3Cl_5O$	278	130	1
240	Methyl myristate	$C_{15}H_{30}O_2$	242	74	1
332	Alkylated aromatic		190	105	
311	A methylpentadecanoate, probably methyl n-pentadecanoate	$C_{16}H_{32}O_2$	256	74	1
343	Appears to be aromatic		232	232	
312	Methyl palmitoleate	$C_{17}H_{32}O_2$	268	55	1
244	Methyl palmitate	$C_{17}H_{34}O_2$	270	74	1
304*	3,6-dimethylphenananthrene	$C_{18}H_{14}$	206	206	1
346	Carboxymethyl derivative		244	185	
315		$C_{18}H$	294	67	1
316	Methyl oleanate	$C_{18}H_{34}O_2$	296	41	1
317	Isomer of methyl olearate	$C_{18}H_{34}O_2$	296	55	1
246	Methyl stearate	$C_{18}H_{36}O_2$	298	74	1
349	Aromatic		322	117	

*Internal standard.